

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE
ENGINEERING AND TECHNOLOGY

**DEVELOPMENT OF MOLECULARLY IMPRINTED HYDROGELS FOR
DRUG RELEASE SYSTEMS**



Ph.D. THESIS

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Department of Polymer Science and Technology

Polymer Science and Technology Programme

SEPTEMBER 2017

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

**İLAÇ SALINIM SİSTEMLERİ İÇİN MOLEKÜLER BASKILANMIŞ
HİDROJELLERİN GELİŞTİRİLMESİ**

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To my husband and son,



FOREWORD

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ABBREVIATIONS

MIP	: Imprinted Polymer
NIP	: Non-Imprinted Polymer
IF	: Imprinting Factor
DSC	: Differential scanning calorimetry
DPC	: Differential Photo Calorimetry
FTIR	: Fourier transform infrared spectroscopy
SEM	: Scanning Electron Microscope
EWC	: Equilibrium Water Content
AA	: Acrylic Acid
IA	: Itaconic Acid
HEMA	: 2-Hydroxyethyl Methacrylate
EGDMA	: Ethylene Glycol Dimethacrylate
TEGDMA	: Triethylene Glycol Dimethacrylate
DOX	: Doxycycline Hyclate
MTT	: Cell viability assay to test the cytotoxicity
RPE	: Retinal pigment epithelium
PBS	: Phosphate buffered saline
PMS	: Electron coupling
XTT	: 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide
4-VP	: 4-vinyl pyridine
Dk	: Oxygen Permeability
DVB	: Divinyl benzene
MMA	: Methyl methacrylate
DMF	: Dimethyl Formamide
TRIM	: Trimetholpropane trimethacrylate
AIBN	: Azobisisobutyronitrile
UV	: Ultra Viole radiation
CL	: Contact Lens
DEAEM	: (2-diethyl amino)ethyl methacrylate
PEG200DMA	: Polyethylene Glycol 200 Dimethacrylate
NO	: Nitrous oxide
CNV	: Corneal Neovascularization
HPLC	: High Performance Liquid Chromatography



SYMBOLS

ΔG	: Gibbs Free Energy
Q	: Adsorption Capacity
C_0	: Initial Concentration
C_e	: Final Concentration
K	: Rate Constant
T_g	: Glass transition temperature
n	: Release exponent
k	: Release rate constant
ξ	: Mesh size
$\text{\AA}$	: Amstrång
L	: Film thickness of the hydrogel
D	: Diffusion coefficient
v	: Specific volume of the polymer
V_1	: Molar volume of water
χ_{12}	: Polymer-water interaction parameter
M_n	: Average molecular weight of the polymer chains
$\bar{M}_c$	: Molecular weight between two crosslinks
M_r	: Molecular weight of repeating unit
τ	: Tortuosity of the matrix and
K_H	: Higuchi constant
M_t / M_∞	: Fraction of drug released at time t
$V_{2,s}$	: Volume fraction of the hydrogel
V_p	: Polymer volume
V_s	: Swollen gel volume
ρ_p	: Polymer density
ρ_s	: Solvent density
m_0	: Dry hydrogel weight
m_s	: Swollen hydrogel weight
C_n	: Flory characteristic ratio
l	: Length of the bond along the polymer backbone
N	: Number of links per chain
K_0	: Zero order release constant (concentration/time).
C_s	: Drug solubility in the matrix
T_g	: Glass transition temperature



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DEVELOPMENT OF MOLECULARLY IMPRINTED HYDROGELS FOR DRUG DELIVERY SYSTEMS

SUMMARY

In the past few decades, hydrogels have been a focal point of many researches in the biomedical and pharmaceutical fields. They offer great promise for applications in cell therapy, tissue engineering and as vehicles for controlled drug release. Recent studies in the area of molecularly imprinted hydrogels in controlled release of drugs highlighted these materials as a promising platform for controlled ocular drug delivery systems.

The purpose of this study was to fabricate novel Doxycycline Hyclate (DOX)-imprinted ocular controlled release hydrogels by establishing a relationship between material structure and its performances. The material-property relationship was established and used to predict the releasing performances of the DOX-imprinted hydrogels. Initially, a systematic method was designed to investigate the parameters affecting the fabrication of MIPs by thermal and UV induced polymerization. Effectively optimization of the polymerization conditions including reaction type, initiator concentration, reaction period and reaction temperature resulted in high degree of monomer conversion. Design of experiments (DOE) as a systematic approach to engineering problem-solving was applied using MINITAB to ensure the generation of valid and defensible conclusions from the model parameters. The model parameters used in the pre-polymerization were comprised of crosslinker type, crosslinker ratio, monomer type, monomer ratio and drug ratio. Pre-polymerization interactions between template (DOX) and model parameters were investigated via UV measurements and binding studies.

In the dissertation, statistical methods were also adopted to releasing pattern of DOX-imprinted hydrogels. Statistical analysis was performed with ANOVA (Analysis of Variance) with the data obtained from the drug release results. Four different releasing criterias were used to obtain regression models separately. The advantage of regression model lies in its flexibility regarding decision making process in controlled release hydrogel formulation prior to experimentation. Running the models obtained from statistical analysis played a key role in reduction of time and number of experiments. Furthermore, the obtained models facilitated optimization of the desired properties of the formulations for the subsequent synthesized materials. Response optimizer was used to decide the optimum formulation based on the desired properties. Based on the predicted optimum formulations new hydrogels were synthesized and the obtained results were compared with the predicted ones. The results showed that the predictive formulations obtained with the experimental design were consistent with the experimental results.

Both imprinted and non-imprinted hydrogels were characterized by Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC).

Morphological differences between non-imprinted and imprinted hydrogels were clarified by Scanning electron microscope (SEM) analysis. Structural analysis were conducted by NMR analysis.

The effects of modelling parameters on release and loading performances of both imprinted and non-imprinted hydrogels were investigated experimentally. In this stage, interactions between the variables were explained via non-covalent chemistry. Then, based on the release performances, drug release kinetic profiles were evaluated. The structural and mass transfer properties of molecularly imprinted and non-imprinted hydrogels were explored for controlled drug delivery applications. Swelling behaviors of all hydrogel systems were studied in loading solution, releasing medium and distilled water. Then, the swelling behavior of the hydrogel systems were used to determine their structural properties. The determination was based on the calculation of volume fraction in swollen state ($V_{2,s}$), the mesh size (ξ), and the average molecular weight between crosslinks ($\bar{M}_c$) of the hydrogels. Thereafter, to obtain a more careful design of DOX-imprinted hydrogels along with network design, mathematical approaches were used to determine drug transport mechanisms of all hydrogels. The Mathematical models were used to fit the release data in order to gain insight into the release mechanism. For this purpose, Korsmeyer-Peppas and Higuchi Equations was used to understand the transport mechanism of drug molecules. Korsmeyer Peppas with the largest regression coefficient was determined as the best-fit model. The non-imprinted hydrogels had higher Korsmeyer release exponent (n), indicating generally super case II transport. The DOX-imprinted hydrogels had relatively low n values and therefore, exhibited non-Fickian transport mechanism, in general.

According to loading and release performances, DOX-imprinted hydrogel samples which included IA and TEGDMA, displayed great controlled drug release performance compared to the other hydrogels. According to this results, the hydrogels were synthesized in both high and low crosslinker ratios with IA-TEGDMA and their performances were compared with the non-imprinted counterparts. DOX-imprinted hydrogels with high crosslinker ratios provided highly effective controlled release performance than the others.

This thesis study showed that experimental studies successfully used with statistical modelling methods. At the same time, the parameters affecting the properties of the imprinted hydrogels have been successfully defined and their interactions with each other has been examined. The results of the study showed that each imprinting system exhibited different characteristics due to different affinities towards template molecule. Experimental and statistical studies could be used in a meaningful way and these studies contained informative information for studies with a low crosslinker ratio in the absence of solvent during polymerization. Finally, the thesis study will be followed by a mutli-disciplinary approach by biologists, physicians, chemical engineers and chemists in order to develop contact lens and perform in vivo studies for the treatment of neovascularization via TUBITAK supports (Project No:114M459).

İLAÇ SALINIM SİSTEMLERİ İÇİN MOLEKÜLER BASKILANMIŞ HİDROJELLERİN GELİŞTİRİLMESİ

ÖZET

Hidrojeller, biyomedikal ve farmasötik alanlarda yapılan birçok araştırmanın odak noktasıdır. Hücre terapisi, doku mühendisliği ve kontrollü ilaç salınımı gibi uygulamalarda kullanmak için büyük bir potansiyele sahiptir. Sentezde kullanılan kimyasalların çeşitliliği ve geniş yelpazeye yayılan fiziksel ve kimyasal özellikleri nedeniyle oldukça dikkat çekmektedir. Moleküler baskılanmış polimerler de son yıllarda üzerinde oldukça fazla çalışma yapılan bir alandır. Hidrojellerin baskılanmış polimer olarak çalışılması da yaygındır. Kontrollü ilaç salımı günümüzde ilaçtan etkin şekilde yararlanmak ve zararlarını en aza indirmek için üzerinde önemle durulan bir araştırma alanıdır. Kontrollü ilaç salımında moleküler baskılanmış hidrojellerin kullanımı son 10 yıldır ilgi görmektedir. Özellikle bu malzemeleri uzun süreli göz içi hastalıklarının tedavisinde ilaç dağıtım sistemleri olarak kullanmak umut verici bir alan görülmekte ve üzerine çalışmalar yoğunlaşmaktadır.

Bu tez çalışmanın amacı, Doksisiklin hidrat (DOX) ilaç molekülünü kalıp molekül olarak kullanarak kendine has özellikleri olan neovaskülarizasyon tedavisinde kullanılacak yeni moleküler baskılama tekniği ile üretilmiş kontrollü ilaç salımı yapan hidrojeller hazırlamaktır. Bu amaçla baskılanmış polimerlerin nihai özelliklerine etki eden temel parametreler değişken olarak belirlenip, ilaç salım ve yükleme özelliklerine olan etkileri belirlendi. Malzeme ve özellik ilişkisi kurularak daha sonra yapılacak olan ihtiyaca uygun DOX-baskılı hidrojellerin performanslarını tahmin edebilmek amacıyla deneysel tasarım yapıldı. Bu yöntem klasik deney yöntemlerinin aksine az sayıda deneyle çok fazla değişkenin irdelenmesini ve belli bir cevaba göre modellenmesini sağladı.

İlk yapılan çalışmalarla hidrojellerin üretim metodu belirlenip sabitlendi. Isı ve ışık ile polimerizasyonlar gerçekleştirilip reaksiyon hızına ve dönüşüme etkileri incelendi. Işığın ilacın yapısına zarar verebileceği hatta reaksiyon hızına ve monomer dönüşümüne ciddi etkilerinin olduğunun anlaşılması üzerine hidroje sentezine ısıl yöntemle polimerizasyon ile devam edilmesine karar verildi. Isıl polimerizasyonda hidrojel özelliklerini etkileyen parametreleri araştırmak için sistematik bir deney seti tasarlandı. Başlatıcı konsantrasyonu, reaksiyon süresi ve reaksiyon sıcaklığı dahil olmak üzere polimerizasyon parametrelerinin etkin bir şekilde optimizasyonu yapılarak, monomer dönüşümünün yüksek olduğu optimum şartlar belirlendi. Mühendislik problemlerini sistematik bir yaklaşım ele alarak çözmeye yarayan MINITAB aynı zamanda deney setlerinin belirlenmesinde yani deneysel tasarımın yapılmasında etkin şekilde kullanıldı. Moleküler baskılanmış polimerlerin özelliklerini etkileyen bir çok parametre vardır. Bunlardan yukarıda bahsedilen 4 tanesi ön deneylerle sabitlendikten sonra değişken olarak model parametreler belirlenerek deneysel tasarım yapıldı. Bu parametrelerin belirlenmesinde literatürdeki çalışmalarda kullanılan değişkenler ve bu değişkenlerin baskılanmış polimerin özelliklerine olan etkileri göz önüne alındı. Model parametreleri; çapraz bağlayıcı türü, çapraz bağlayıcı

oranı, monomer türü, monomer oranı ve sentezde kalıp molekül olarak kullanılan ilaç oranıydı. Deneysel tasarım sırasında kısmi faktöriyel tasarım metodu kullanıldı ve toplamda 20 baskılı ve karşılaştırmak için 20 baskısız hidrojel deneysel tasarım sonuçlarında elde edilen formülasyonlara uygun sentezlendi. Model parametreleri arasındaki reaksiyon süresince meydana etkileşimlerin hidrojel performansına etkileri ilaç salım ve yüklenme özelliklerinin belirlenmesi yoluyla yapıldı. Bu amaçla UV-Vis spektrofotometresi ilacın konsantrasyonunun belirlenmesinde kullanıldı.

Bu doktora çalışmasında, DOX-baskılı hidrojellerin ilaç salım performansının modellenmesi ve irdelenmesi için istatistiksel yöntemler benimsenmiştir. Sonuçların modellenmesi ve etkilerinin belirlenmesi bizim için önemli 5 cevabın ilaç salım sonuçlarından elde edilen verilerle hesaplanması yapıldıktan sonra analizi edilmesi ile gerçekleştirildi. Her cevap için ANOVA (Varyans Analizi) ile ayrı ayrı regresyon denklemleri kuruldu; toplamda 4 adet olan bu regresyon denklemleri sayesinde modellemede minimum hatanın olması hedeflendi. Deneysel modellemenin avantajı, deneyden önce ilaç salımı yapan hidrojel formülasyonlarının belirlenmesi üzerindeki esnekliğidir. İstatistiksel analizden elde edilen modellerin kullanılması zaman ve deney sayısının azaltılmasında anahtar rol oynamıştır. Dahası, elde edilen modeller, sonraki sentezlenen malzemeler için istenilen özelliklerinin optimizasyonunu yapmak için çok önemli birer tahmin aracı olarak kullanıldı. İstenilen özelliklere dayanarak optimum formülasyonu belirlemek için yanıt iyileştirici kullanıldı. Bu amaçla 4 yanıtı optimize edilmesi istenenlerin değerleri belirlenip sisteme girildi ve tahmin edilen optimum formülasyonlara dayanarak yeni hidrojeller sentezlendi. Sentezlendikten sonra performansları belirlenen hidrojellerin modellemedeki kriterlere karşı cevapları tahmin edilenlerle karşılaştırıldı. Deney sonrasında hesaplanan sonuçlarla deneysel tasarım sonucunda belirlenen formülasyon için tahmin edilen sonuçların tutarlı olduğu görülmüştür ve bu sayede de deneysel modellemenin baskılanan hidrojellerin tasarlanmasında kullanılabilir olduğu ispatlanmıştır.

Baskılı ve baskısız hidrojellerin her ikisi de Fourier transform infrared spektroskopisi (FTIR) ve diferansiyel tarama kalorimetresi (DSC) ile karakterize edildi. Baskısız ve baskılı hidrojeller arasındaki morfolojik farklılıklar Taramalı elektron mikroskopu (SEM) analizi ile açıklığa kavuşturuldu.

Baskılı ve baskısız hidrojellerin serbest bırakma ve yükleme performanslarına modelleme parametrelerinin etkisi deneysel olarak araştırıldı, karşılaştırıldı ve yorumlandı. Bir sonraki aşamada, değişkenler arasındaki etkileşimlerin nedenleri ve sonuçları klasik kimya yoluyla açıklandı. İlaç salım performanslarına dayanarak, ilaç salım kinetik profilleri değerlendirildi. Kontrollü ilaç salım uygulamaları için moleküler baskılanmış ve baskılanmamış hidrojellerin yapısal ve kütle transferi özellikleri araştırıldı. Tüm hidrojel sistemlerinin şişme davranışları, yükleme çözeltisi, serbest bırakma ortamı ve damıtılmış su içinde incelendi. Bu yapılan çalışmalar neticesinde ortam pH' ının ve aynı zamanda tuz oranının şişme davranışına etkisinin büyük olduğu belirlendi. Daha sonraki yapılan hesaplamalarla yapısal özelliklerini belirlemek için hidrojellerin şişme davranışlarının belirlenmesi oldukça önemliydi. Yapısal özelliklerin tayininde ve karşılaştırılmasında şişmiş haldeki hacim fraksiyonu ($V_{2,s}$), ağ boyutu (ξ) ve hidrojellerin çapraz bağları arasındaki ortalama moleküler ağırlığı ($\bar{M}_c$) hesaplanmaları yapıldı. Bundan sonra, ağ yapısının belirlenmesi ile birlikte DOX-baskılı hidrojellerin ilaç salım performanslarının daha dikkatli bir incelemesi için, ilaç salım profillerinin matematiksel yaklaşımla incelenmesi yapıldı. Salım mekanizması hakkında fikir sahibi olmak için, matematiksel yaklaşımlar oldukça önemlidir ve yaygın şekilde kullanılır. Bu amaçla, Korsmeyer-Peppas ve

Higuchi yaklaşımlarını deney sonuçlarımıza uyguladık. Korsmeyer Peppas yaklaşımı ile modellenen veriler en büyük regresyon katsayısına sahip olduğundan esas model olarak tespit edildi. Baskısız hidrojellerin çoğunun “super case II” taşınım mekanizması ile ilaç salınımı gerçekleştiğini işaret eden daha yüksek Korsmeyer salınım üssü (n) vardı. DOX ile baskılanmış hidrojellerin n değerleri nispeten düşüktü ve genel olarak Fickian olmayan transport mekanizması ile ilaç salınımı yaptıklarını ortaya koyuyordu. Bu durum baskılamanın ilaç salım mekanizmasını değiştirdiği ortaya koydu. Aynı zamanda elde edilen sonuçlara göre baskılı hidrojellerin baskısız olanlarla karşılaştırıldıklarında daha fazla miktarda ilacı serbest bıraktığı belirlendi. Yükleme ve salım performanslarına göre, IA ve TEGDMA'yı içeren DOX-baskılı hidrojel örnekleri diğer hidrojellere kıyasla kontrollü ilaç salım performansları sergilemiştir. Bu sonuçlara göre, hidrojeller, IA-TEGDMA ile sınır koşullarındaki durumların tespiti için hem yüksek hem de düşük çapraz bağlayıcı oranlarında sentezlenerek performansları baskılı olmayan muadilleriyle karşılaştırıldı. Yüksek çapraz bağlayıcı oranlarına sahip DOX-baskılı hidrojeller, düşük çapraz bağlayıcılı hidrojellerden daha etkili bir şekilde kontrollü salım performansı sağladı.

Bu tez çalışması, deneysel çalışmaların istatistiksel yöntemlerle başarıyla modellenebileceğini göstermiştir. Aynı zamanda baskılı hidrojellerin özelliklerini etkileyen parametreler başarıyla tanımlanmış ve birbirleriyle etkileşimleri incelenmiştir. Yapılan çalışmalar klasik deneysel yöntemlerden farklı olmakla birlikte oldukça kullanışlıdır. Çözücüsüz ortamda gerçekleştirilen çalışmalar için mekanizmayı aydınlatıcı önemli bilgiler içermektedir. Deneysel tasarım ve analizler neticesinde belirlenen formülasyonların sonuçları tahminlerle karşılaştırıldıktan sonra kontakt lens üretimi ve TÜBİTAK ile yapılan in vivo çalışmalar için kullanılacaktır.



1. INTRODUCTION

People has been using materials to aid in improving the human condition since earlier times. Early examples of the use of gold in dental fillings, and of using wood for prosthesis for severed organs are evident from anthropological history [1,2]. However, it took until the turn of the 20th century for advancements in materials and chemical engineering, as well as our understanding of relationships between materials and their effect on the body and vice versa to develop sufficiently to allow for the field of "biomaterials" to truly develop. Biomaterials are generally described as any material used in a medical device, but new examples of non-medical applications of biomaterials such as DNA microarrays and cell culture platforms suggest that the common definition may be too narrow [1,3]. The success of biomaterials used in humans has passed through various applications such as scaffolding for the growth of new bones, stents for keeping narrow vessels open, and dental implants instead of missing teeth. Negative home-agent interactions initially prevented medical supplies from being successfully used [3]. The use of organic materials such as wood as medical devices has inevitably given undesirable immunological reactions by the body and has caused a device failure [1]. The success of modern biomaterials has seen insights into the design, synthesis, and implementation of materials with appropriate material-material interactions.

The use of intraocular lenses (IOLs) after cataract extraction surgery, where an artificial lens is implanted into an eye in place of the removed, cloudy, natural lens, provides a useful example of the properties needed for successful biomaterials. Success of a biomaterial depends on two distinct, broad properties. First, it must be somehow accepted by the body. The material cannot cause an immunologic reaction that results in persistent inflammation or a host immune response or the material will eventually be rejected. The material also cannot be toxic to the area of the body where it is to be used. Second, the biomaterial must achieve a function in some way [3].

In the majority of cases, a function of the biomaterial will be defined by the disease that is being treated [4]. These two defining features of biomaterials are described in

the broadest terms available to illustrate the fact that what is needed will necessarily be disease and location specific. In the design of IOLs, for example, the choice of material such as acrylates, hydrogels and silicone were chosen not only because they had favorable host-material interactions with the eye, but also because they could be manufactured with the desired optical properties to correct vision [5]. Properties that facilitate success within blood vessels, where desirable properties may include prevention of blood clot formation, and stability in enlarging the blood vessels. These properties may not be applicable for biomaterials used in the mouth for example, where the toughness of the material against wear and corrosion are more important [1]. Consideration must also be made for the timeframe of use of the biomaterial, as materials that are intended to be used indefinitely within the body may have required properties different than those that will be removed at a later date. Materials used forever within the body have to be carefully studied for any long term adverse effects, and maintenance of desired function. For example, while one may think hip replacement surgery to be an indefinite operation, the lifespan of the materials in this very mobile joint is only 10-15 years [6]. Initially, the aim of biomaterials was biological inertness in conjunction with their intended function. Materials were chosen that would not interact biologically with the host organism. The majority of these early materials served a more mechanical support type of role. Titanium and titanium alloys for orthopedic applications are a prime example of an early biomaterial whose main biological aim was inertness [4]. Titanium alloys benefitted from having strength and structural rigidity to aid in the healing of broken bones, with the added benefit of being corrosion resistant and invisible to the immune system for years after implantation [4]. In contrast, as the field of biomaterials has continued to evolve, newer materials are being engineered to positively interact and affect the course and management of diseases in addition to mechanical support. An example of these newer materials have been introduction to the market of heart stents which release compounds to prevent vessel re-stenosis, and thus improve recovery time after a cardiac ischemic event [1]. The materials still have a mechanical function in keeping the blood vessel open, but now they also can release factors to encourage the body to behave in a therapeutically beneficial way.

A molecularly imprinted polymer is a synthetic polymer having selective molecular recognition properties due to recognition sites that are complementary to the template

molecule in the polymer matrix for the dimensional positioning of functional groups [7]. A number of experimental variables should be optimized in order to develop of molecularly imprinted polymers which define the thermodynamic balance of the system and affinity of functional monomers to the template molecule. The selection of functional monomers according to the nature of the template, the ratio of monomer to template (M:T), the degree of crosslinking and the polymerization solvent are the most substantial parameters that must be carefully optimized [8].

Molecularly imprinted polymers (MIPs) have been extensively studied for high cross-linked and solvent-containing systems to achieve polymer template complexation. Although the molecular imprinting technique is commonly used for special polymer synthesis, very few study has been advanced to clarify the factors that drive the imprinting process to predict MIP performance for low crosslinked and non-solvent systems. An important step in polymer synthesis is the pre-organization phase in which a complex is created between the template and a functional monomer or a series of functional monomers. Today, it is clear that if this complexation is more stable or stronger, MIP is more selective. Understanding the physical and chemical rules principal of the monomer-template complex formation is essential to rationally designing polymerisation systems with high selectivity and affinity. If we understand the significant parameters (i.e. polymerization process, washing step, monomer and crosslinker selection, monomer and crosslinker moiety identification etc) that regulate the formation of intermolecular interactions, we can better understand the polymer recognition properties.

The robustness and ease of preparation of MIPs as well as the selective recognition feature made them attractive not only to analytical chemistry but also to a wide range of applications in drug delivery areas [9,10]. Today the activity of the molecular imprinting on the drug release is being studied and the diversity of the drugs used for imprinting has a wide range from antibiotics to chiral drugs. Nicholls and coworkers conducted the first study about using MIPs as drug delivery carriers [11]. They synthesized theophylline-imprinted polymers and investigated the drug dissociation kinetic, at different pH and loaded with different amounts of drug. Their affinities were compared with caffeine and it was observed that theophylline-imprinted polymers demonstrated higher affinity towards theophylline than to caffeine. And also, imprinted polymers released theophylline more sustained than non-imprinted

counterparts. Many other reports were published in order to develop MIPs with potential use as DDS after this study. Some recent developments include preparation of materials for ocular, transdermal and oral drug delivery by using molecular imprinting [8].

The templates consist of theophylline [12,13], [14], propranolol [14–19], citalopram [20], H1-antihistamines [21], sulfasalazine [22], 5-Fluorouracil [23,24], norfloxacin [25], hyaluronic acid [26], levofloxacin [27,28], timolol maleat [29–32], ketotifen fumerate [33] have been successfully used. The real big challenge in MIP synthesis is the imprinting of macromolecules, charged or low functionality molecules [36].



2. AIM AND MOTIVATION

Molecular imprinting is a technique that can be used for molecular recognition by forming template-like cavities in the polymer structure. Molecular imprinted polymers (MIPs) are a comprehensive research topic that exists over a period of 20 years. One of the earliest publications on the imprinting of molecules is related to imprinting of solvent molecules onto silica gels by Polyakov in the 1930s [1,2]. Later, In the 1970s and 1980s, Wulff and Mosbach groups developed the basic concepts and terminology of MIP technology and led to the continuous development of research activities in the molecular imprinting field.

MIPs are unique materials with a wide range of potential applications in chromatography, sensors, diagnostic devices and controlled drug delivery areas. Intensive research has been done in the field of molecular imprinting. Previous studies are concerned with synthesis methods of MIPs, binding parameters and level of affinity to template and application of the MIPs to the existing technologies. These studies have been mostly conducted experimentally and small number of them supported by statistical methods.

The objective of this dissertation was to develop molecularly imprinted polymers using non-covalent imprinting technique and investigate their performance in controlled drug release due to neovascularization treatment. Doxycycline hyclate (DOX) was chosen as template molecule. Since the solvent was not used in the synthesis of the molecularly imprinted hydrogels, the crosslinker ratio was therefore maintained low compared to the solvent systems. Non-solvent imprinted hydrogel systems constitute only a small fraction of the studies on polymer-imprinted polymers. The relationship between variables (crosslinkers, monomers and their ratios and template ratio) and material properties were investigated and model parameters were then selected, models were predicted in order to simulate releasing. It was decided to apply a five-part methodology to explain the model parameters and their effect on the performances of the imprinted polymers. In the first part, variables are selected, binding ability

experiments were completed and data is gathered for clarification of the interaction of the variables with template molecule. In this part, monomers and crosslinkers evaluated separately and also together with certain combinations. In the second step, the experimental design technique is used as a powerful method in order to optimize MIPs rapidly and attain desired performances. The number of experiments and the contents of the imprinted hydrogels (MIP) were determined using MINITAB according to the limit values given. In order to be able to compare each of the imprinted polymers, a control counterpart was synthesized without using the template as non-imprinted hydrogel (NIP). In the third part, in vitro release data is collected for both NIPs and MIPs designated via experimental design method. The studies are used to explain the impact of the hydrogel properties on the release mechanism kinetics. The fourth part empirical models are fitted to release profiles in order to recognize a release mechanism and identify kinetic parameters. The fifth part includes the grouping of model parameters and noticing relationships between the model parameters and the varied material properties of the release system via General Linear Model (GLM). Determination of these relationships is significantly important to better understand the effects of design factors on drug release kinetics of imprinted and non-imprinted hydrogels. In the literature, there are no published studies that have worked on this scope with non-solvent imprinted polymers for drug releasing.

Recently, studies on the inhibitory effects of doxycycline on ocular neovascularization have increased. DOX which is selected as template molecule, is a charged drug molecule and a broad-spectrum inhibitor of matrix metalloproteinase (MMPs). Many studies have demonstrated that oral doxycycline strongly inhibits MMP activity and effectively treats ocular neovascularization. However, the use of topical medicines is generally a more appropriate treatment for corneal diseases. Some studies have been governed with doxycycline-containing hydrogels and have shown effective results in the treatment of neovascularization. Molecular imprinting technique is a successful technique in controlled drug release applications and there had not been published any study about the treatment of corneal neovascularization with molecularly imprinted hydrogels.

The use of statistical analysis methods to propose model will benefit the decision-making process in the controlled release DOX imprinted hydrogel formulation by allowing simulation of the release before trial. Presenting a model greatly reduces the

time and effort required for DOX release imprinted hydrogels systems. The model or models may also be used for optimization to determine optimal hydrogel formulation conditions that will tailor the release rate to suit the needs of the release application. This is called as response optimization and can be used to expand the prediction capabilities of new DOX imprinted hydrogel systems for controlled release by investigating more design factors and considering one or more responses from kinetic models. In addition, restrictions of the release from these imprinted hydrogels can be defined, by identifying a DOX-imprinted hydrogel release mechanism and understanding the mass transport processes involved. This information will help future research by producing new formulation ideas to overcome these restrictions.





3. THEORETICAL STUDY

3.1 Molecular Imprinted Polymers

The first studies of molecular imprinting technique were discussed in literature at 1930s by Soviet Chemist M. V. Polyakov research group [37]. They reported that the solvent molecules (benzene, toluene, xylene) could be imprinted to silica gels and this solvent was chosen selectively for rebinding if it was available during gel synthesis [38]. This event showed that there was a selective adsorption due to application of imprinting method [39]. At 1949, Dickey imprinted methyl orange and established that silica gels had great affinity for dyes, which is available during gel synthesis. He also showed that binding capacity of the gels had increased with imprinting technique [40]. Wulff et al. published a study which is the first recommended processing of an imprinted polymer that may have specific selectivity to a racemic mixture of glyceric acid in 1972 [41]. In this study, it was reported that two different approaches for interaction between template molecule and monomers; covalent and non-covalent. In Wulff's study, molecular imprinted polymer (MIP) was selective to enantiomers of glyceric acid by covalent (covalent bonding between template molecule and functional monomer) approach [42]. In 1981, non-covalent approach was adopted to MIP manufacturing (chiral drug separation). Afterwards, it was reported that combination of covalent and non-covalent approach was possible and have some advantages for MIP. Even so, the most preferred approach for molecular imprinted polymer synthesis is non-covalent approach today [38].

3.2 Principle of Molecular Imprinting

In general principle, monomers that have functional groups interact with template molecule covalent or non-covalently. Rigid polymers are produced with crosslink network in the presence of functional monomer and template. The template molecules are cleaned from polymer structure and empty cavities are obtained. The cavities in the polymer structure whose shape, dimension and coordination of the binding sites

that interact with template molecule are settled with respect to the structure of template molecule [7].

Accordingly, molecular imprinting has fundamentally two essential scholarly regions: molecular recognition and polymer chemistry. Whereas molecular recognition frequently takes attention in the imprinted polymer manufacturing, the point of fact that this recognition must occur in the polymer not be escaped from the attention [39]. Therefore, it is required to consult fundamental of polymer chemistry. Methodologies for production of molecular imprinted polymers will be discussed in more detail in Section 3.3.

3.3 Methods for MIP Synthesis

The interaction between functional monomer and template is the key parameter for accomplishment template-monomer complex and also achievement necessary functionality in the polymer structure. This interaction has an important effect on the implementation phase of the polymer. Interaction type is selected according to not only template but also the application of the final product. There are three main ever-growing strategies to synthesis molecular imprinted polymer with suitable functional monomer-template interaction. These strategies are covalent, non-covalent and semi-covalent approaches and they will be discussed below.

3.3.1 Covalent approach

Template and functional monomer make a reversible covalent bond. At this stage, the template-monomer complex is fixed by polymerization, high crosslinker ratio is used and polymerization is completed in a solvent medium. To remove template, polymer must be treated chemically such as hydrolysis due to break bond between template and functional monomer. Chemical bond is composed when re-binding is required [39].

As mentioned before, the first imprinted polymers were synthesized by covalent approach by Wulff and Sarhan [43]. Divinyl benzene (DVB) was used as crosslinker and D-gliseric acid was used as template molecule.

The template-monomer complex was created in polymer structure, the the template was removed from the polymer by hydrolysis and D-glycemic acid was rebind to the binding sites successfully. Thus, L-glycemic acid was not bind to functional groups on

the polymers due to be improper alien of functional groups. The cavities could be cleared from template and rebinding capacity is equal to this amount.

Another example for covalent approach is MIP synthesis for specific sugar or amino acid derivatives which contained a polymerizable functional group such as vinylphenyl boronate. After polymerization sugar was hydrolyzed and the polymer was used for selective binding. The results showed that selectivity of MIP increases with increasing crosslinker ratio for covalent molecular imprinting [44].

Covalent imprinting has several advantageous: The adequate ratio of functional monomer to template obtain specific binding sites and non-specific binding probability decreases. Resolution of the template-monomer complex is remained during polymerization. Binding sites distribute homogeneously.

On the other hand there are drawbacks about covalent approach: The template-functional monomer complex is obtained with limited amount of the functional monomer and template. Low binding site is achieved. The washing of the template is adverted as a trouble [45]. Template releasing and rebinding events complete slowly (slow rebinding is a disadvantage for chromatography). These disadvantages are causes of using semi-covalent or non-covalent approaches for several applications [39].

3.3.2 Non-covalent approach

The non-covalent approach is the most preferred approach in order to create molecularly imprinted polymers which the functional monomers arrange around the template molecule by non-covalent interactions. The interaction between monomer and template occurs via hydrogen bonding, ionic interaction, hydrophobic effects, dipole-dipole bonding and π - π interactions. The template-monomer complex is relatively less stable than the covalent approach. The variety of the possible interactions that constitute template-functional monomer complex, permits to select different type of templates. On the other hand, many type of functional monomers are available due to forming imprinted polymer with non-covalent approach [44–47].

Non covalent approach was firstly used by Mosbach and Arshady. They work with dye and create a term as “substrate-selective polymer”. Rhodamine blue and safranine O were used as templates [48]. The monomers that were used to synthesize imprinted polymer are methyl methacrylate (MMA) and methylene diacrylamide. H₂O:DMF

mixture used as solvent mixture and reaction completed at 40 °C. The binding and selectivity properties of imprinted polymers were compared with the polymer which was synthesized without template molecule (it is called as non-imprinted polymer). The results showed that binding capacity and the selectivity were achieved by imprinting process. The studies about non-covalent approach causes to increase incrementally the range of functional monomers, the number of templates and application areas. The most commonly used templates in molecular imprinting technology with non-covalent approach are proteins, antibiotics (sulphones, micellous etc), aminoacids (cormark, cartridges, rick myoglobin, malosse, ekberg) [39]. The strategy of the non-covalent approach is represented in Figure 3.1.

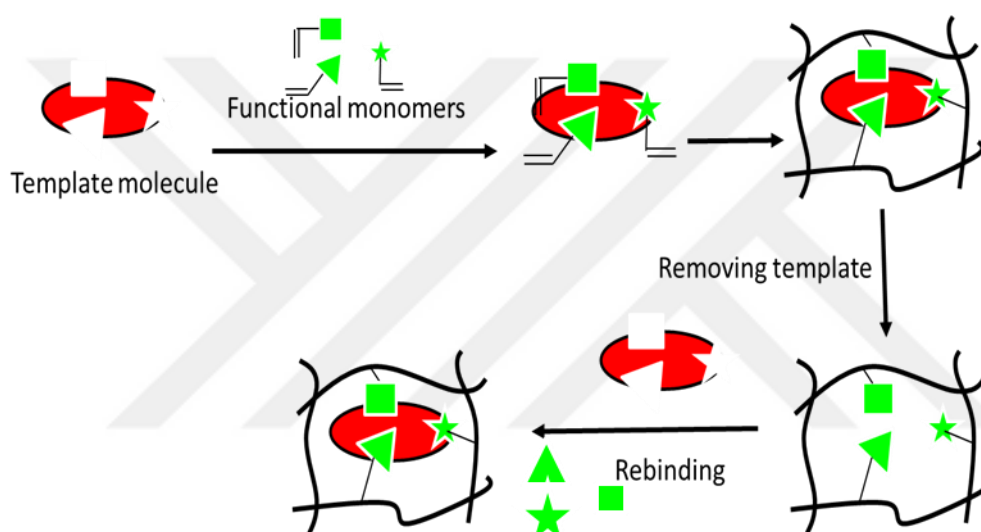


Figure 3.1 : Schematic representation of non-covalent imprinting method.

Firstly, the monomers associate around the template molecules according to their hydrogen bonding abilities. The monomer-template complex is obtained when the required time is provided. Crosslinker and initiator are added to polymerization mixture and synthesis is completed. The template is removed by using washing solvents and then polymer is used to rebind template in cavities that has ability to form non-covalent interactions. Methacrylic monomers, such as methacrylic acid, acrylic acid is used commonly in order to create non-imprinted molecularly imprinted polymers because its carboxyl group is the most common hydrogen-bonding and acidic functional group in molecular imprinting. Additionally, mostly ethylene glycol dimethacrylate (EGDMA) is preferred as crosslinker in this approach. But, some studies showed that molecularly imprinted polymers prepared with the trifunctional crosslinkers such as pentaerythritol triacrylate and trimethylolpropane trimethacrylate

(TRIM) were shown to be superior to those prepared with EGDMA, in that higher load capacities and better resolution were obtained [49]. Also, studies with long-chain crosslinkers yield better results than EGDMA were published. For example, Jiang et al. studied ketoprofen on the purpose of (R) (S)-ketoprofen separation [50]. A chiral monomer was synthesized and it acted as both a crosslinker and a functional monomer. The monomer had three functional group that could make π - π bonding, H-bonding and ionic interaction. The final product was utilized for HPLC column successfully. When two or more interaction type is used together, the material shows biomimetic property of the molecular imprinting method.

There are many advantageous of non-covalent approach. Numerous templates can be imprinted. There are wide ranges of functional monomer that can be used for molecular imprinting. The rebinding rate is faster than covalent approach. Non-covalent approach includes the vast array of solvents. Several type of initiation mechanism can be used to synthesize molecular imprinted polymer without harming template molecule [51].

On the other hand, heterogeneity of the binding site is encountered frequently. The excess of monomer is used to achieve monomer-template complex when the monomer template interaction is poor [52]. These cause to employ more complex but many of them are low affinity binding sites [53].

3.3.3 Semi-covalent approach

Binding sites with homogeneity nature is constituted by covalent approach but rebinding is succeed applying non-covalent approach. With the other words, binding sites are created by covalent interaction but rebinding is observed by non-covalent approach. Rebinding capacity with covalent approach is larger than rebinding capacity with non-covalent approach because of electrostatic interaction [43].

Andersson and Sellergen used semi-covalent approach to synthesize p-aminophenyl alanine ethyl ester imprinting for the first time via semi covalent approach [54]. Ester linkages that have two polymerizable group reacted covalently with template. When reaction was completed, imprinted polymer that have two carboxylic acid groups rebinded aminoacids via non-covalent binding. Semi covalent imprinting was used for imprinting propazine [55]. Template and monomer is reacted together and template

was removed from polymer via hydrolysis after completion of reaction. The polymers made ready to bind again propazine non-covalently

3.3.4 Stoichiometric approach

For this approach, the functional monomer is selected according to magnitude of affinity between template and monomer. Therefore, it is not necessary to use excess of the functional monomer due to achieve high affinity binding sites [39,45,52]. Nowadays, the monomers are synthesized according to template molecule structure, privately.

This approach especially is used to prepare catalyst imprinted polymers, although it is applied to solid phase extraction [56]. Advantages of this method are fast rebinding rates, sensitive position of the functional groups in the polymer structure and high removing yield after polymerization.

3.4 Optimization of the Polymer Structure for Favorable Application via Non-Covalent Approach

Molecular imprinting can be adjusted to several type of target molecule especially by non-covalent approach. The target molecules separate from each other with their polar properties, solubility, functional groups, biocompatibility properties, etc. The application area of the molecular imprinted polymer requests some properties from MIP product. There are several polymerization methods for imprinting polymer and factors that affect the final product properties. Herewith, optimization of the reaction conditions and components that create the structure of final product is very significant for the best progress [39].

The progress of MIPs embodies the optimization of the several factors that affect the thermodynamic balance of the system and creation of the optimal structure for best recognition. The more substantial factors are monomer type, crosslinker type, crosslinker ratio, monomer ratio, porogen and template ratio.

3.4.1 The functional monomer

Functional monomers are responsible for binding interactions in the polymer structure [57]. For non-covalent approach, relatively greater amount of monomer is used than template molecule. It is fundamental that the maximum template-monomer interaction

can be formed by interaction of functionalities both monomer and template. Binding site creation is substantially assignment of the functional monomer in a molecular imprinted polymer. There are various types of functional monomers that have different structures, polarity values and functional groups. Some of them are commercial products. The others are special synthesized products that specify according to template molecule [57]. It is important to choose the true functional monomer due to optimization and enhancing molecular imprinting. In Figure 3.2, the chemical formulations of the most commonly used monomers are represented.

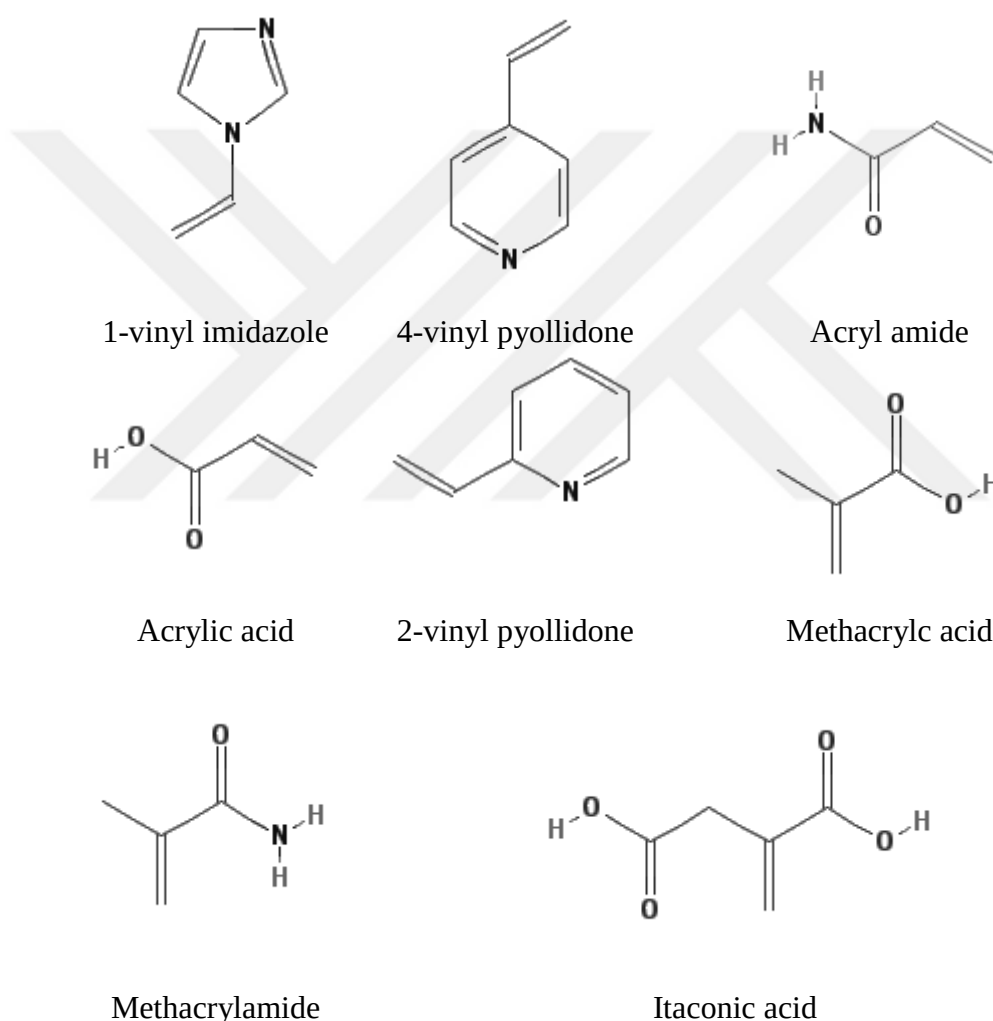


Figure 3.2 : Chemical structures of common functional monomers used in non-covalent imprinting [57].

Karim et al. reported that over 4000 compounds can be used for polymerization as functional monomer [58]. However, a small part of these monomers can be applied for imprinted polymer formation. As mentioned in Section 3.3, especially non-imprinting approach allows to use more monomer type for formation of molecular imprinted

polymers. These monomers are mainly classified as neutral, acidic and basic monomers [39,53,57].

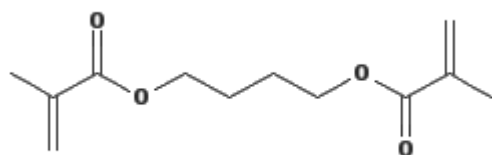
Some monomers can be applied for the same template while molecular imprinted polymer synthesis. For instance, 4-vinyl pyridine and MAA had been used to imprint of 17 β -estradiol [59,60]. In the study, monomer performance was compared for the 17 β -estradiol imprinted polymers. The results showed that MAA had better developed binding capacity than 4-VP due to hydrophilic structure of the monomer. It was reported in that study, the choice of the monomer is a key factor for imprinted polymer performance.

Scientists obtained that combining two or monomer may help for better performance for MIP. 2-Hydroxyethyl methacrylate (HEMA) is one of the most popular neutral monomer that is combined with different type of monomers such as AA, 4-VP, MAA, MMA [29,61–67]. Janiak et al. used negatively and positively charged monomers in order to prepare MIPs. The specific property of the polymer was increased but selectivity results were adversely affected because of the negative charged monomer [68].

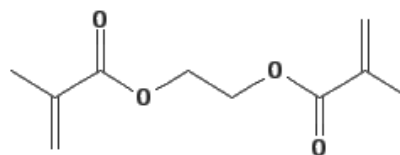
When two or more monomers are used for preparation of MIPs, it is called as cocktail polymer [69]. If the cocktail polymer is synthesized, another important parameter about monomer selection is reactivity ratios of the monomers. If the reactivity ratios of the monomers are different, all monomers polymerize at different rates. Hence, polymer structure is not as expected; distribution and composition of the monomers will depend on the monomer concentration and this will change with time [57].

3.4.2 The crosslinker

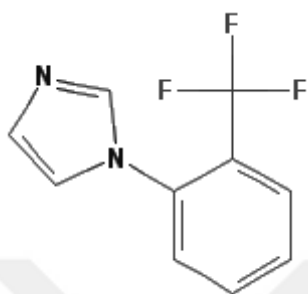
Crosslinkers connect two or more linear polymer chains and non-linear polymers are prepared. The selectivity is greatly affected by the type and ratio of crosslinker used in the preparation of the imprinted polymer. The crosslinker should be chosen carefully in order to provide complementary interactions with the template and functional monomers. There are large number of crosslinker monomers that can be used with monomers comfortably. The chemical structures of the most commonly used crosslinkers are represented at Figure 3.3.



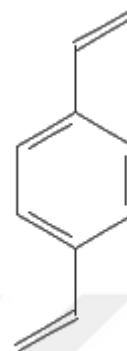
Tetra methylene dimethacrylamide



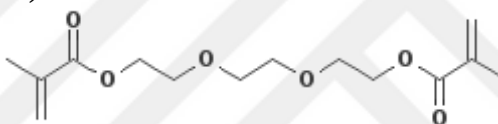
Ethylene glycol dimethacrylate (EGDMA)



Trimethylolpropane trimethacrylate (TRIM)



Divinyl benzene (DVB)



Triethylene glycol dimethacrylate (TEGDMA)

Figure 3.3 : Chemical structures of common crosslinkers used in non-covalent imprinting [57].

The crosslinkers are used for three main purposes. Firstly, the morphology of the polymer is regulated by crosslinker monomers. Secondly, it associates the binding site of the polymers. Thirdly, mechanical properties of the resultant polymer depend upon crosslinker monomers [57]. Non-linear polymers are synthesized in molecular imprinting technique. Because of that molecular imprinted polymers are insoluble in water and in other solvents. There is few research about soluble linear imprinted polymers but major of the studies are related on crosslinked polymers. The amount of crosslinker should be enough in order to retain molecularly binding sites stability. Then highly crosslinked imprinted polymers which were synthesized in the solvent were preferred [44]. On the other hand, for the non-solvent systems relatively low amount of crosslinker is used for solvent free MIP synthesis.

Divinyl benzene (DVB) was used to prepare molecular imprinted polymers for D-glyceric acid selectivity over L-glyceric acid by Wulff and Sarhan [43]. The study was related to comparison of five crosslinkers (including DVB and EGDMA) for

separation of a racemic solution. According to the results, EGDMA was the most influential crosslinker monomer (70-95 %) for this racemic solution.

Afterwards, EGDMA became the most popular crosslinker in the molecular imprinting studies [18,29,49,54,65,70,71]. Many studies have been carried out to investigate the effect of the crosslinker ratio on imprinting. For instance, Sellergren stated that the separation factor of the polymer during chromatographic measurements attained a supreme value at 75 % EGDMA, however below this value (such as 50 %) selectivity of the polymer was lost [72]. More recent studies support this study with their results; high degree of crosslinker was important for molecular imprinted polymer selectivity. Holland et al. showed that specificity of the polymer was absent when the EGDMA concentration was decreased in the polymer composition [73].

Although, some studies claimed that high crosslinker monomer ratio is required for effective selectivity. In MIPs, many studies have proved that it is not necessary for some applications. The work of Alvarez-Lorenzo et al. is an example; the soft contact lenses prepared by molecular imprinting technology showed selectivity which the functional monomer:crosslinker ratio was 1:10 [74]. Hilberg et al. prepared successfully theophylline selective membranes with 12.5 % triethylene glycol dimethacrylate (TEGDMA) [12].

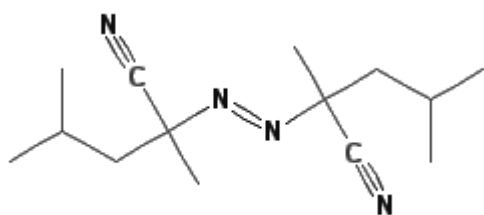
The dipeptides and steroids were studied by Mosbach. Results showed that TRIM have proven to be more effective than EGDMA as a crosslinker in molecular imprinted hydrogel [24,75]. Cirillio et al. used ethylene bis acrylamide as crosslinker for imprinting antioxidant, phytic acid, in 3:1 water:ethanol mixture as porogen [76]. The use of more hydrophilic crosslinkers was necessary due to the hydrophilic nature of the template in order to use polymer in a controlled release application. The final products release drug completely in 2.5 hour for NIPs while MIPs release only 40 % drug at acidic fluid for the same period.

Careful selection of the crosslinker is also essential. Even though the effect of crosslinker and interaction with template was disregarded generally, the reactivity ratio of the crosslinker monomer and functional monomer must be matched. Solubility and compatibility factors must be taken in consideration [39].

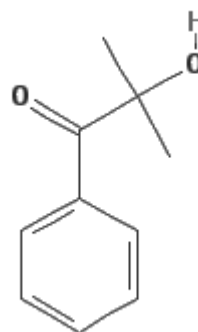
3.4.3 Polymer initiation

There are many methods for initiating polymerizations such as γ -irradiation [77], use of iniferter [78], using peroxide based initiators [79]. Most widely used method is to use azo-derivative initiator and their decomposition products to generate radical species. Azobisisobutyronitrile (AIBN) [27,80,81], 2-methyl propiophenone and bis(2,4-dimethyl valeronitrile) (Vazo® 52) [15,82,83] are the most popular initiators for MIP preparation.

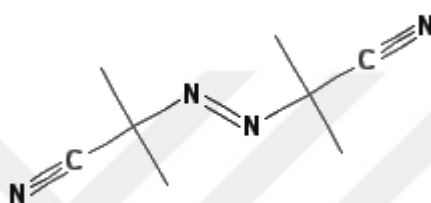
These azo initiators decompose by photochemically and/or thermally and form radicals. Photochemical polymerization allows to study at relatively low temperatures which the interaction between template and monomer could be supported positively. Zhang et al. and Dopico et al. published a study about comparison initiation systems [84,85]. Zhang et al. used AIBN initiator as both thermally and photochemically and compare the pore size of the polymers for non-imprinted polymer (NIP) and MIP [86]. The study proved that the pore structure of photo-initiated polymers are different when MIP and NIP were compared. This difference between the NIP and MIP was considered to be important for the performance of the polymer. The selectivity and adsorption capacity was relatively high than thermally initiated polymers. Generally, photo initiation is preferred because of low temperature of reaction conditions. But, there can be advantages over one thing to another. For instance, if the template is photo-sensitive and decomposes easily under light, thermal initiation systems will be preferred. Conversely, if template is unstable when the heat is applied, the photo initiators are triggered. Hydrogen bond strength is strong at lower temperatures and therefore photochemically active initiators are selected [57]. But nowadays, some crosslinkers such as Vazo® 52, are utilized at low temperature (45 °C) for thermal polymerization. The chemical structures of selected initiators are shown in Figure 3.4.



2-2'-Azobis(2,4-dimethylvaleronitrile)



2-Hydroxy-2-methylpropiophenone



Azo-bis-isobutyronitrile (AIBN)

Figure 3.4 : Chemical structure of initiators used most commonly in molecular imprinting [57].

3.4.4 Solvents

The first function of the solvent is to bring together all the component of the polymerization mixture (template, functional monomer, crosslinker monomer, and initiator) into a single phase [57]. To bring all components together before polymerization, has an significant role on the formation of template-functional monomer complex. The second function is to generate macro pores on the polymer matrix. Hereby, the solvents are called as porogen for molecular imprinting systems [39,57]. The morphology/nature of the pores, pore volume can be controlled by the level of porogen. Thermodynamically good solvents provide to lead to polymers with well-developed pore structures and high specific surface area. Thermodynamically poor solvents tend to lead to poor developed pore structure and low specific surface areas. The pore volume increases with increasing solvent volume [57].

3.5 Polymerization Methods and Techniques

Molecularly imprinted polymers can be produced via a range of polymerization methods according to its application area. The polymerization strategies that is adapted to imprinting technique have been discussed in earlier sections for imprinted polymers. The notable advantages and disadvantages of the polymerization strategies were summarized in Table 3.1.

Table 3.1 : Advantages and disadvantages of the polymerization strategies [39.

Technique	Advantages	Disadvantages
Bulk monolith	Easily optimized Suitable for most polymer formulations	Irregular particue size Low yield Time intensive
Suspension	Cotrollable particule size Spherical particles	Surfactant necessary
Precipitation	Controlable particule size Spherical particles No stabilizer necessary	Needs optimization for each set of monomers
Core-shell	Controlable particule size Spherical particles	Stabilizer necessary Multiple polymerization step
Thin film	Well suited for special applications	Slow diffusion Poor thickness control

While the strategies are summaries according to their advantages and disadvantages, application area and its requirements have not been considered. Whatever the strategy used, physical properties of the final product is important due to understand the behavior of the final product when it is utilized to an application. Here, some common polymerization methods that have been utilized for MIP production will be summarized.

3.5.1 Bulk polmerization

All polymerization components which are soluble in solvent medium are mixed together. Concentration of components must be 40-60 % of total volume [75]. Final product is intensely fused and bonded microgels. This product is then crashed, ground and sieved in order to obtain required size. The first imprinted polymers have been prepared by this method and it has been the most widely used method for preparation of MIPs [45]. Bulk polymerization is simple and practicable although the polymer is lost in a large extent (up to 70 %) due to grinding and sieving processes. And also this causes destroying of the binding sites of the polymer.

3.5.2 Molecularly imprinted beads

Beads from molecular imprinted polymers can be produced by several techniques. Most widely suspension and precipitation techniques are used. Spherical particles are considered for some applications such as chromatography [87] and drug delivery [22].

3.5.3 Suspension polymerization

Typically, suspension polymerization method is applied to molecular imprinted polymerization. Basically water insoluble monomers are dispersed in the chosen solvent like droplets. Each droplets act as a mini-bulk reactor [45]. The first studies about suspension polymerization for non-imprinted hydrogels have been carried out by Mayes and Mosbach [88]. The dispersing phase was a liquid hydrocarbon (Perfluorocarbon) and UV initiation system has been used to initiate polymerization. The bead sizes were varied between 5-50 μm . The template monomer interaction has been obtained successfully for this system. Mineral oil was used as dispersant and acetonitrile was used as solvent by Mayes and Mosbach. Beads which have been produced by this method had approximately same sizes with beads of Mayes and Mosbach [89]. This made the process cheaper and does not need stabilizer. Stabilizers generate undesirable functional groups on the polymer surface. Although these genuine methods have been used to prepare molecular imprinted polymers, classic suspension methods are still popular. Triazine, melamine [90], peptide hepcidine [91] and Sudan dyes [92] are templates that were used to prepare molecular imprinted polymers via classical suspension methods.

3.5.4 Precipitation polymerization

The monomers are soluble in the solvent and take up <5% to all volume in diluents solvent. When the polymerization start, the insoluble growing polymer chains collapse and generate polymer beads [93]. The size of the beads is varied in the of 0.3-10 μm stabilizer does not put in the polymerization mixture. And also, if the proper solvent is chosen, the morphology and size distribution of the beads can be controlled. Theophylline and 17 β -estradiol imprinted beads have been produced by this method by Mosbach et al. with excellent rebinding ability over the bulk monolith particles [75]. Warfare agents [94], anticonvulsant [95], antimalarial [96] and antipsychotic drugs [97] have been imprinted by using this method.

3.5.5 Core-shell MIPs

Core-shell method overcomes some disadvantages of the other techniques such as dilution problems of spherical MIP beads. Perez et al. reported that this process have two significant steps; firstly a core is generated by emulsion polymerization of the monomers. At the second stage, this core is supplemented to another batch of monomers. Thin imprinted shell is formed on the surface of the core. The advantages of this method is to easily remove template from imprinted polymer, binding is relatively fast, good control of the bead size, be prepared larger beads.

3.5.6 Film and lens type MIPs

This method is required to produce of imprinted polymers in film and lens type such as sensors and contact lenses. The templates that have been used to produce sensors for targeting analysts such as myoglobin [98], bilirubin [99] and melamine [90] were studied. Hiratani et al. have utilized polypropylene and Teflon to build molds due to develop molecular imprinted contact lenses in desired thickness [100–102]. The biocompatibility of the monomers and polymers are significantly important for these kind of applications.

3.6 Applications of Molecularly Imprinted Polymers

The durability and the stability under hard conditions of the molecular imprinted polymers allows to use them in many applications [39]. Molecular imprinted polymers has been utilized in many field such as sensors [103–106], solid phase extraction [107–110], chiral separation [9,46,50,111,112] and catalysis [53,113,114]. However, the application of them in drug delivery has gain attention by studies of Norell et al. and Sreenivasan et al. related on the controlled release from MIP matrices [77,115]. The potential of MIPs in drug delivery application has been explored with development of drug delivery systems as intelligent therapeutics [116]. It has been suggested that molecular imprinted polymers have a significant potential for drug delivery systems [38].

3.6.1 Contact lens drug delivery systems

The advantages of using eye drops and ointments for topical treatment of ocular disorders over other forms of treatment center around the location and accessibility of

the eye [117]. The anterior segment of the eye is readily accessible externally, and thus treatment with eye drops and ointments can directly target the eye and surrounding structures [118]. Frequent dosing can allow for relatively high concentrations of the drug to be reached within the ocular tissues fairly easily, as long as the patient is compliant [119]. By applying the treatment directly to the ocular tissues, the hepatic first pass effect can be bypassed compared to orally administered agents. The chief challenge to effective eye drop therapy are issues with patient compliance. Often, the dosing frequency may be too difficult to manage for patients with acute conditions. During treatment of chronic conditions even once a day dosing can be very difficult to adhere to 100 % of the time [120,121]. Eye drops have also been shown to be a relatively inefficient means of delivering drugs to the eye [122]. Computer modeling and measurements estimate that only between 1-7 % of the active ingredient within an eye drop is able to overcome all of the ocular barriers and exert a therapeutic effect within the eye [118,123]. This is clearly economically and therapeutically disadvantageous, in order to drugs do not reach the site of action and are wasted and unnecessary. Current eye drops, thus, achieve appropriate concentration profiles by utilizing highly concentrated solutions instilled frequently [124]. The volume being instilled from a commercial eye drop bottle is also significantly larger, at 35-56 μL , than what can be reasonably accommodated by the ocular surface which has a theoretical maximum capacity of 30 μL , and routinely only has 7 μL of tears on it [124,125]. The excess fluid overflows out of the eye and spreads across the patient's cheek. Approximately 75 % of the instilled drop which is retained by the ocular surface is removed by the nasolacrimal duct, with the remaining 25% being lost to the conjunctival vasculature [126]. The pharmacokinetics of eye drops are not ideal. Eye drop pharmacokinetic profiles are characterized by pulsatile delivery, with only a short period of time within the therapeutic window, surrounded by periods of either overdose or underdose [117]. Finally, as mentioned previously, treatment of the eye through the anterior segment is also unable to affect the posterior segment [4,125]. Ophthalmic ointments partially alleviate some of the disadvantages of eye drops by significantly improving ocular residence time [127]. The ointment vehicle is often a petroleum or jelly-like substance with low water solubility, and thus when placed on the ocular surface is much more difficult to be flushed away through actions of the tears or blinking [4]. The major disadvantage of ophthalmic ointments is their unfortunate side effect of impairing vision. The thick ointment causes a significant

blockage of light transmission, such that they are generally only useful for overnight use when the visual function of the patient is not a primary concern [124].

Use of contact lenses (CL)s to serve as a drug reservoir for drug delivery to the anterior segment of the eye is not a new idea. Sedlacek first proposed the idea in 1965, and this was followed by further postulations in the 1970s by Gasset, Kaufman and Waltman [25,26,28,128]. The theory was that the CL could serve as a depot for the therapeutic agent, decreasing the number and frequency of administrations, and potentially increasing the drug's effectiveness by improving drug penetration or improved drug residence time [129,130]. There are also several commercially available CLs (balafilcon A, lotrafilcon A, etafilcon A) which have a special designation by the FDA for "therapeutic use", that is, they can be employed in situations primarily to aid in the management of diseases or disease process within an individual, rather than for the correction of ametropia. The FDA categorizes "therapeutic use" of CLs into applications for pain relief, the promotion of wound healing (such as after a corneal abrasion), providing mechanical support (such as post traumatic injury), maintenance of corneal hydration (in severe dry eye cases) and drug delivery [129]. The use of lenses as a drug delivery device is underutilized, while the first four applications are routinely seen and used in clinical practice when indicated. The main goals in investigating CLs as a drug delivery platform are to improve ocular bioavailability, improve patient compliance, decrease drug wastage and prevent undesirable side effects. In this application, the CL can be loaded with the drug by soaking within a drug solution before lens insertion, or combined with eye drop instillation over the top of an already worn lens. There exists some evidence that simply by wearing a CL on the ocular surface during drop instillation increases drug penetration, with one study suggesting that diffusion into the cornea may be up to five times higher [129]. Indeed, mathematical modelling suggests that up to 50 % bioavailability from a drug releasing CL, versus the 1-7 % seen with eye drops alone [131,132]. CLs would thus provide an ideal platform for treatments which require a large amount of drug to enter into the anterior segment. Investigations into the concurrent use of CLs and antibacterials, anti-inflammatories, an anti-allergy eye drops found that they offered increase corneal and aqueous humour concentrations when compared to the use of CLs alone. The increase in drug penetration and bioavailability would have a direct effect on the efficiency and rates of adverse reactions systemically, as more of the active agents reach the intended

tissues [131]. The concentration of the solution used to load the lenses could also be decreased due to better bioavailability [133]. The effect on patient compliance is unknown, but is speculated to have a positive effect due to the dual nature of CLs in drug delivery applications. By coupling the delivery of needed medication to the eye, which can be easily ignored for many diseases which have little or no symptoms until the latter stages of the disease (such as glaucoma), to the correction of refractive error, which provides a real, immediate and tangible benefit to the patient, it is expected that patient compliance can readily be improved [131]. Simplification of drug treatment regimens have also been shown to improve patient compliance, which is particularly important if one wishes to prevent the development of bacterial resistance in the use of antibiotics [117]. CL manufacturing is also well established on a large, industrial scale, which would drive the unit cost of each one of these lenses down [131]. Finally, the biocompatibility and complications of CL wear are well known, and there is comfort in both prescribing eye care practitioners and patients alike in the use of CL technology [130]. Insertion and removal of CLs is relatively easy, ensuring that they will both be used appropriately, and can be withdrawn quickly if they are causing any complications. When surveyed, eye care practitioners have indicated that they would use a CL drug delivery device, should one be available [134].

3.6.2 Drug delivery from MIPs

The direct synthesis of molecularly imprinted drug delivery materials is achieved by using hydrogels. The properties of the hydrogels are discussed at above. Water absorption properties of the hydrogels make suitable them for practice in biological applications. There are several research related on molecular imprinted hydrogels that have been studied for controlled drug release [25,33,100,135–138].

The first study was published for potential use as soft contact lens of imprinted hydrogels in 2002 [74]. Timolol imprinted hydrogels were synthesized due to treatment glaucoma. The imprinted hydrogels reduced the ocular pressure inside the eye and prevent ocular nerve damage. Methacrylic acid (MAA) or methyl methacrylate (MMA) was used as functional monomers with hydroxy ethyl methacrylate (HEMA). Imprinted hydrogels released timolol in 6-10 hours in artificial lacrimal solution via Fickian transport mechanism. The thickness of the hydrogel $\sim 700\text{ }\mu\text{m}$, it was thicker than conventional lenses and also thicker films delays drug transport.

Another study which was published by the same authors contained timolol imprinting using MAA as functional monomer with different crosslinker ratios and different backbone monomers [100]. It was found that there was a minimum crosslinker concentration for effective imprinting as well as the effect of backbone monomer structure and hydrogel swelling on recognition. 300 μm or greater thick films were tested and they released 90 % of timolol in 16 h [100] and 4 h [29]. When the release curves of imprinted and non-imprinted hydrogels were compared, it was observed that there was no differences in the shape of the curve. And also, there was no significant differences in diffusion coefficient between imprinted and non-imprinted hydrogels.

The other study made a correlation between affinity and release rate. It was found that increasing the functional monomer to template (M:T) ratio within the hydrogel, decreased drug release rate [101]. With this study 50 % drug was released in 3 days.

The M:T ratio is a significant variable in an effective molecularly imprinted polymer design. There is a substantial evidence for optimal M:T ratio in highly crosslinked imprinted polymers. Weakly crosslinked polymers also have an optimum M:T ratio. The first works were published by Alvarez-Lorenzo and co-workers [25,101]. In the non-covalent systems, the reversible monomer-template interaction is achieved by using excess of functional monomer. Then, M:T ratios are not commonly in stoichiometric amounts based on the number of template functional. This optimum can be clearly seen by looking at two extreme cases. At a very large excess of M:T ratio, the specifically distributed monomer within the polymer chains is very small compared with the randomly distributed monomer and there is no difference between the non-imprinted and imprinted hydrogels. At lack of M:T ratio, multiple monomer interactions with the template is not effective, resulting in no recognition.

The first study which a high molecular weight template imprinted in a hydrogel was hyaluronic acid imprinting study. Nelfilcon A, acrylamide (AM), N-vinyl pyrrolidone (NVP) and 2-(diethylamino) ethyl methacrylate (DEAEM) were used as monomers and films were synthesized in $\sim 120 \mu\text{m}$ thickness in the presence of HA. As a result, imprinted hydrogels released HA over 24 h [26]. The diffusion coefficient of 1.2 million dalton HA was controlled by changing the number of functional monomers (using more than one function monomer lowered the diffusion coefficient 1.5 times more than single functional monomers, and 1.6 times more than nelfilcon A alone) [26,139].

Anti-inflammatory, diclofenac sodium, imprinted hydrogels were prepared by living polymerization mechanisms [139]. Poly(DEAEM-co-HEMA-co-PEG200DMA)-imprinted gels synthesized by means of 'living/controlled' polymerization techniques exhibited a 54% increase in loading capacity over poly(DEAEM-co-HEMA-co-PEG200DMA)-imprinted hydrogels synthesized using conventional free-radical polymerization. Release results showed that imprinted hydrogels synthesized via living polymerization extends drug release by two fold over than imprinted hydrogels synthesized via conventional polymerization and four fold over that of non-imprinted hydrogels.

Hiratani and Alvarez-Lorenzo have prepared successfully hydrogel contact lenses for controlled drug release for timolol and norfloxacin as templates [25,100]. The ocular therapy is one of the application area of molecular imprinted polymers. The poor patient compliance and inadequate bio-availability of ophthalmic drugs are the well-known disadvantages. Molecularly imprinted hydrogels have been synthesized which release antibiotics, ciprofloxacin during a long time. When the non-imprinted and imprinted hydrogels loaded with relatively lower concentrations, imprinted hydrogels showed better release properties [64]. Thus, these hydrogels enhance patient compliance about administration of eye drop.

Javanbakht et al. prepared molecular imprinted polymers of dipyrindamole. The MIPs showed slower release rate than NIPs. MIP released drug completely in 65 hours [103].

3.7 Doxycycline hyclate and its structural properties

Tetracyclines are wide spectrum antibiotics which prevent protein synthesis by binding bacterial ribosome [140]. Tetracyclines are widely used in treatment of human and animal infections. Because, they have unfavorable adverse effects and appropriate antimicrobial characteristics [140]. The tetracyclines are developed from soil bacterium *Streptomyces aureofaciens* [141].

The members of the tetracycline antibiotics are chlortetracycline, oxytetracycline, tetracycline, rolitetracycline, limecycline, clomocycline, methacycline, doxycycline, minocycline, and tertiary-butylglycylamido minocycline [140]. Despite a few of them are present as parenteral form, generally tetracyclines are implemented as oral passage, (oxytetracycline, Limecycline, Doxycycline, Minocycline). Tetracyclines that are used

for ocular treatment are generally are focused on ophthalmological findings in both kids and adults [141].

Doxycycline is long-acting, semi-synthetic member of tetracycline family. It is available in three forms: hydrate salt, hyclate (hydrochloride solution in methanol and water) and monohydrate. Usually, hyclate form is used as drug. Monohydrate form is insoluble in water. Hydrate form is usually used as hyclate form. It is a metal ion chelator [142,143] and divalent metal ions (Mg^{+2} , Mn^{+2} , Co^{+2} , Ni^{+2} , Cu^{+2} , Zn^{+2} , Cd^{+2}) can improve its stability [144]. And also it is used as oral form or parenteral form. Doxycycline inhibit matrix metalloproteinase (MMP) synthesis, interleukin-1 synthesis, activated B cell function, nitrous oxide (NO) synthesis by lipopolysaccharide (LPS) activated macrophage collagen synthesis by cultured chondrocytes doxycycline a role in ocular surface [145–149].

Studies show that preclusion of enzymes that endanger cornea structure, may an alternative treatment for corneal neovascularization (CNV) [150]. Doxycycline are utilized for treating CNV as a non-selective MMP inhibitor with topical corticosteroids [150].

Doxycycline has been researched comprehensively as a well-known MMP inhibitor and has been approved in clinical implementation and in vivo studies [151–153]. Doxycycline blocks the growth of MMPs and prevent the corneal destruction by the reason of chemical damage and other corneal ulcerations that are not related with infections [154–156]. Improving of the cornea is supported by doxycycline. It can be used as oral and topical medicine as before mentioned. Previous studies had been showed that doxycycline which is used as oral form is influent on the CNV healing [157–160]. On the other hand, the several researches about doxycycline solution as topical applications had been explored for CNV healing. As a result, 2% neutralized doxycycline solution adequately hinder CNV for a rat that had been injured chemically burning [157,159,161,162].

To escape from the side effect of the drugs and use them effectively, topical method is an optimal method for corneal healing [163]. Temperature sensitive hydrogels (DTSH) were prepared by Su et al. and results showed that inhibition of the MMPs successfully improved and the patient were preserved from side effect of the clinical doxycycline application [157]. Another study about topical doxycycline usage is related to

polyethylene glycol hydrogels. These hydrogels are ability to heal CNV effectively due to mustard injuries by inhibiting MMP synthesis [164].

DOX is one of the salt forms of doxycycline [165] which displays zwitterionic character generated by the carboxylic acid moiety and the protonated amine. Above all, it is important to know that many intramolecular H-bonds can form in the DOX molecule and its incorporation into the cavities during monomer-template complexation may allow them to disappear or perhaps cause the revelations to appear [166]. The intramolecular hydrogen bonding positions can be seen in Figure 3.5.

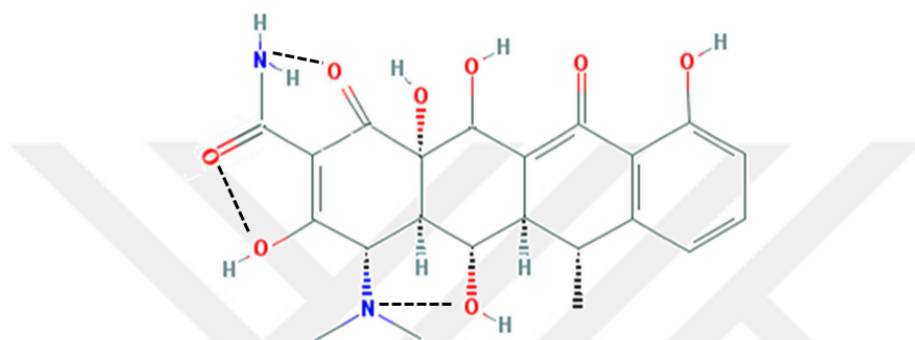


Figure 3.5 : The intramolecular hydrogen bond positions for DOX.

Doxycycline structural feature leads to the formation of various intermolecular H-bonds between DOX and related monomers [165,167]. There are six groups on the DOX structure which are possible locations for hydrogen bonds and they are marked on Figure 3.6.

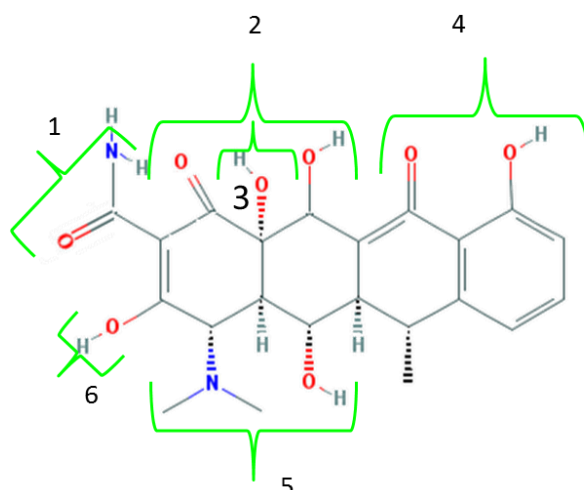


Figure 3.6 : Intermolecular interaction positions for DOX.

Doxycycline acts as both a hydrogen acceptor and hydrogen donor by interacting with hydrogen and oxygen atoms of carboxyl/ether groups of the monomers and

crosslinkers. Acrylic acid and itaconic acid have one carbonyl (-C=O) and one hydroxyl (-OH) group and two carbonyl (-C=O) and two hydroxyl (-OH) groups, respectively (Table 4.1). There are two possibilities. One of the possibilities is that intramolecular hydrogen bonds cause to reduce the ability of this hydroxyl group to form hydrogen bonds with functional monomers. One of the other possibilities is to be hydrophobic interaction between DOX and hydrogel components because of the non-polar groups on the both monomers (backbone monomer, crosslinker monomer and functional monomer) and DOX. Intermolecular interactions, such as hydrogen bonding, electrostatic interactions and hydrophobic interactions are the main players of energetic stabilization of the most favorable form [166].



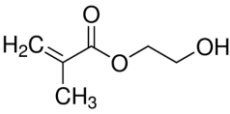
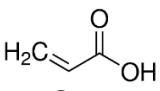
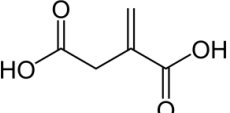
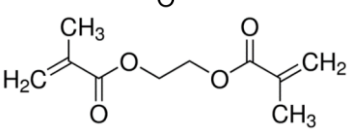
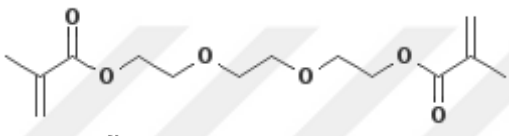
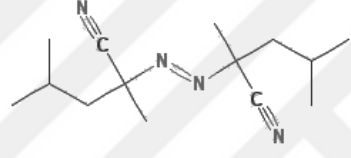
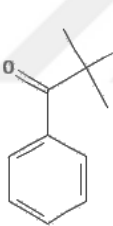
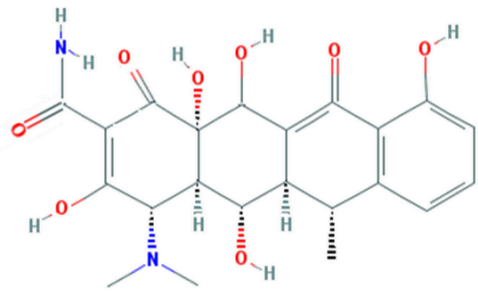


4. EXPERIMENTAL PART

4.1 Material

Commercially available 2-hydroxyethyl methacrylate (HEMA), (contains ≤ 250 ppm monomethyl ether hydroquinone as inhibitor, 97 %) was purchased from Sigma Aldrich. Ethylene glycol dimethacrylate (EGDMA, contains 90-110 ppm monomethyl ether hydroquinone as inhibitor, 98 %) and triethylene glycol dimethacrylate (TEGDMA, contains 80-120 ppm MEHQ as inhibitor, 95%) as crosslinkers were all purchased from Sigma Aldrich. Functional monomers; acrylic acid (AA, anhydrous, contains 200 ppm MEHQ as inhibitor, 99 %) and itaconic acid (IA, ≥ 99 %) were purchased from Sigma Aldrich. 2-Hydroxy-2-methyl-1-phenyl-propan-1-one, Sigma-Aldrich, 97%) and 2,2'-azobis(2,4-dimethylvaleronitrile) (Vazo® 52, DuPont) were used as photo initiator and thermal initiator, respectively. Doxycycline hyclate (DOX) used as template molecule, was kindly provided by Deva (Istanbul, Turkey). Chemical structures of all components are shown in Table 4.1. All chemicals were used as received. Acetonitrile (≥ 99.8 %), oxalic acid dihydrate (extra pure) were purchased from Merck Millipore. Technical grade of Methanol was used. Acetic acid (ReagentPlus®, ≥ 99 %) was supplied from Sigma Aldrich. Sodium chloride (99.9 %) was purchased from Merck for preparing of pellets and it was used after drying in oven for one night at 120 °C.

Table 4.1 : Chemical structures, molecular formulations and molecular weights of the components.

Component	Structure	Molecular weight
HEMA		130.14
AA		72.06
IA		130.10
EGDMA		198.22
TEGDMA		286.32
2-2'-Azobis(2,4-dimethylvaleronitrile)		248.37
2-Hydroxy-2-methylpropiophenone		164.20
Doxycycline hyclate	 HCl 0.5 H ₂ O 0.5 C ₂ H ₅ OH	512.94

4.2 Methods

4.2.1 Design of experiment and statistical analysis

Design of experiment (DOE) is widely preferred experimental design technique on many engineering process. A two-level fractional factorial design was proposed to analyze the effects of five different independent variables: monomer type (A), monomer ratio (mol %) (B), crosslinker type (C), crosslinker ratio (mol %) (D), drug

ratio (mol %) (E). The factorial design was measured on two levels, a lower value and a higher value for each factor analyzed. The levels of independent variables assessed in this study are represented in Table 4.2. The addition of the center points ensures that an independent error estimate is obtained without affecting the estimates of the factorial effects and detect of nonlinearity in the results [168].

It is a kind of statistical method in which a mathematical model is settled through experimental runs. Moreover, it offers opportunity to the users in order to optimize and forecast probable output based on the variable setting [169]. A five-factor 20-run experimental design was constituted using DOE from MINITAB® (Version 17.1.0.0). The software package was used to estimate the response of dependent variables and optimized conditions.

Table 4.2 : Factor levels and values of all variables in 2-factorial design applied during experimentations.

Independent Factors	Parameters	Level and Values		
		Low (-1)	Medium (0)	High (+1)
A	Monomer type	AA	-	IA
B	Monomer ratio (mol %)	1	3	5
C	Crosslinker type	EGDMA	-	TEGDMA
D	Crosslinker ratio (mol %)	1	3	5
E	Drug ratio (mol %)	0.160	0.205	0.250
Dependent Factors				
Y1	Time for 90 % of drug released (h)			
Y2	Amount of drug released in 4 h (%)			
Y3	Total amount of released drug in 48 h			
Y4	n ₁ values obtained from Korsmeyer Peppas equation			

The results obtained from experiments were analyzed by analysis of variance (ANOVA) using MINITAB (PA, USA) statistical software, which allow to clarify both main and interactively effects of input variables on the process results. The influence of a factor was considered noteworthy if the value of critical level (p) was lower than 0.05 and neglected the worthless parameters for p values over 0.05.

4.3 Hydrogel Synthesis

4.3.1 Photo induced polymerization

Molecular imprinted hydrogels (MIPs) were synthesized via free radical photo polymerization. As an example for imprinted hydrogel synthesis; HEMA (as the backbone monomer) and functional monomer (AA) were mixed. DOX was added into the HEMA-AA mixture with two different molar ratios (1.33×10^{-5} and 2.66×10^{-5} mols) at two different amount of initiator (8.74×10^{-5} or 4.23×10^{-4} mols). The backbone monomer, HEMA, is liquid, therefore no additional solvent was used. After the addition of initiator and crosslinker, the mixture was sonicated for 5 minutes and was purged with N_2 for 15 minutes to prevent O_2 inhibition of initiator. Then, the solution was transferred into mold and the polymerization was initiated at 365 nm light source at room temperature.

4.3.2 Thermal polymerization

The similar procedures were followed in UV- and thermal-initiated polymerization reaction varying initiator type as thermal initiator, and after transferring into the mold, the polymer solution was kept at $45^\circ C$ for 24 hours for completion of polymerization. Non-imprinted hydrogels (NIPs) were prepared in the absence of DOX by photo and thermal polymerization methods as control hydrogels. And also IA and AA were used functional monomers and EGDMA and TEGDMA were used as crosslinkers.

4.4 Reaction Monitoring and Hydrogel Characterization

4.4.1 FTIR

For hydrogel characterization, FTIR spectra were obtained using Perkin Elmer FTIR Spectrum One B spectrometer from 650 to 4000 cm^{-1} . For UV-induced polymerization, polymerization reaction was monitored by Fourier transform infrared spectroscopy. In order to prevent inhibition effect of oxygen, FTIR cell was purged with N_2 for 30 min before measurement. The monomer mixture was placed between two transparent NaCl pellets and exposed to UV light. The spectral changes were recorded for maximum 3 hours at intervals. The reaction was monitored by measuring the peak height at 1635 cm^{-1} assigned to C=C stretching vibration. The peak corresponds to -C=O carbonyl bond at approximately 1719 cm^{-1} was used as internal

standard. The light intensity was 20 mW/cm². After the end of irradiation, the pellets were annealed at 40 °C to complete the curing reaction and then the Equation 4.1 was used to calculate conversion.

$$Conversion (\%) = \left(1 - \frac{A_{hydrogel\ 1635\ cm^{-1}}/A_{hydrogel\ 1719\ cm^{-1}}}{A_{monomer\ 1635\ cm^{-1}}/A_{monomer\ 1719\ cm^{-1}}} \right) \times 100 \quad (4.1)$$

where A is height of peak at 1635 or 1719 cm⁻¹ for the reaction mixture. R_p was the overall reaction rate, calculated from FTIR data using Equation 4.2.

$$R_p = -\frac{d[M]}{dt} = \frac{[M_0]*(A_1-A_2)}{A_0*(t_1-t_2)} \quad (4.2)$$

where A_0 was the ratio of the peak heights at 1635 cm⁻¹ to 1719 cm⁻¹ at the beginning of the reaction, A_1 was the height of the peak at t_1 , A_2 was the height of the peak at t_2 , and M_0 was initial molar concentration of all monomers in the reaction mixture.

In the studies of thermal polymerization, the reactions were monitored in order to ensure completion of the reactions. The reaction completion was controlled by following the peak at about 1635 cm⁻¹.

4.4.2 DSC and Photo-DSC

DSC measurements were performed in a cycle, by Perkin Elmer (DSC 4000) instrument for thermal induced imprinted and non-imprinted hydrogels. Nitrogen gas was used for purging at a flow rate of 20 ml/min. Temperature scanning program was started to heat from 30 °C to 250 °C, then was cooled from 250 °C to 0 °C. Samples were kept at 0 °C for 15 min. After isothermal step, they were heated from 0 °C to 250 °C again. Scanning rate was 20 °C/min.

The heat flow of the photo polymerization reaction was recorded as a function of time during the polymerization at isothermal conditions by using Pyris Diamond differential scanning calorimeter equipped with EXFO Omni-CureTM 2000 photo-DSC accessory (DPC, Perkin Elmer) for UV induced polymerizations. The temperature was hold constant at 25 °C and N₂ was purged in order to remove the oxygen from DPC cell. Both, the aluminum pan filled with 8-12 mg of reaction mixture and the empty reference aluminum pan were covered with quartz cover. After the stabilization of the test conditions, the light was turned on for 25 minutes. The area

under the endothermic peak of DPC curve was used to calculate the total heat of reaction.

The heat flow of the reaction mixtures was obtained by DPC and the polymerization rate calculated from Equation 4.3 [170].

$$R_p = \frac{H/W}{\Delta H} \quad (4.3)$$

where R_p was the polymerization rate (s^{-1}), H was the heat flow (mW), W was the weight of the monomer solution (g) and ΔH was the enthalpy of the material (J/g).

4.4.3 Absorbance spectroscopic titration

In order to demonstrate template-functional monomer interactions, absorbance spectroscopic titration test was applied to the DOX mixtures including monomers or crosslinkers with different ratios. The proportions of the components were kept the same as in the polymer condition. The analysis was performed by adding the functional monomer or crosslinker solutions (AA, IA, EGDMA and TEGDMA) in the range of 0.4 to 3.125 mM in a constant concentration of doxycycline hyclate solution (0.1 mM) in ethanol. Monomer:Template (M:T) and Crosslinker:Template (X:T) ratio during the polymerization was designed using these concentrations. The solutions were kept in the reaction temperature (45 °C), and then they were measured at scan mode in order to observe both absorbance and λ_{max} changes during pre-polymerization stage. The control solutions without DOX was used as reference solutions at the same concentrations.

4.4.4 Optimization of washing step

Different combination of solvents which were prepared and examined in 4 sets as. 0.01 M Oxalic acid solution was prepared with distilled water. Then, the solution was mixed with various solvents. The applied washing procedures were carried out in two stages. Hydrogels were immersed into four types of solvent mixture in the first step. The washing procedures are given below.

(Mixture 1) The discs were shaken at 100 rpm for 1 day in a dark bottle in 0.01 M Oxalic acid: methanol:acetonitrile 65:15:20 (v:v:v) solvent mixture. Subsequently, they were transferred into methanol, acetonitrile and acetone, separately and washed out for 1 day. Hydrogels were then removed from solvents and washed with distilled

water. After the second step, the washed hydrogels were dried in an oven at 37 °C for 72 hours.

(Mixture 2) The discs were shaken at 100 rpm for 1 day in a dark bottle in 0.01 M oxalic acid: methanol 65:20 (v:v) solvent mixture. Next, they were transferred into methanol, acetonitrile and acetone, separately and washed for 1 day. Hydrogels were then removed from solvents and washed with distilled water. After second step, the washed hydrogels were dried in a vacuum oven at 37 °C for 72 hours.

(Mixture 3) The discs were shaken at 100 rpm for 1 day in a dark bottle in acetic acid:methanol 1:9 (v:v) solvent mixture. Subsequently, they were transferred into methanol, acetonitrile and acetone, separately and washed for 1 day. Hydrogels were then removed from solvents and washed with distilled water. After second step, the washed hydrogels were dried in a vacuum oven at 37 °C for 72 hours.

(Mixture 4) The discs were shaken at 100 rpm for 1 day in a dark bottle in 0.01 M oxalic acid:acetonitrile 65:15 solvent mixture. Afterwards, they were transferred into methanol, acetonitrile and acetone, separately and washed for 1 day. Hydrogels were then removed from solvents and washed with distilled water. After second step, the washed hydrogels were dried in a vacuum oven at 37 °C for 72 hours.

In the second step, the hydrogels were kept separately in acetonitrile, methanol and acetone solvent over a night. The second step washing was applied only to discs which was washed successfully at the first step without deformation (it was controlled by visually).

4.4.5 Scanning electron microscope (SEM)

Washed and dried samples (NIP and MIP) were stuck to double-sided tape. The tape strips were attached to the platform of SEM (JEOL JSM-6390LV). Both low and high magnification images were obtained using platin coated samples. The images helped to provide a general idea about morphology of hydrogels and identify the structure differences between MIPs and NIPs.

4.4.6 Solid-state nuclear magnetic resonance (NMR)

The solid-state ^{13}C NMR spectra of the hydrogels (including 5 mol % TEGDMA, 3 mol % IA, 0.205 mol % DOX in the presence and absence of polymerization) were

recorded using Bruker Superconducting FT-NMR Spectrometer Avance TM 300 MHz WB. All ^{13}C NMR spectra were recorded without using solvent.

4.4.7 Swelling studies

After being dried at 37 °C for 48 h under vacuum atmosphere, the water absorption of the hydrogel network was determined in similar conditions as those experienced in the drug release experiments. Each gel was weighed and put into 1 mM DOX solution firstly for 24 hours. Subsequently, they were removed from loading solution and the excess of water from the surfaces were wiped gently. They were immersed into distilled water and 0.9 % NaCl solution, separately for 24 h. Swelling ratio of hydrogels was calculated using Equation 4.4.

$$Q = \frac{(W_s - W_d)}{W_s} * 100 \quad (4.4)$$

The pH of distilled water and 0.9 % NaCl solution was adjusted through diluted HCl and NaOH solutions, recorded by pH meter (Citizen 3000). All swelling experiments were performed in triplicate.

4.4.8 Oxygen permeability

Oxygen permeability (Dk) is mainly governed by the equilibrium water content (EWC) in conventional hydrogels (HEMA based hydrogels). The relationship between EWC and oxygen permeability can be calculated by Equation 4.5, which is an empirical Equation [171]:

$$Dk = 1.67 * e^{0.0397 * EWC} \quad (4.5)$$

4.4.9 Structural analysis of the hydrogel networks

In order to investigate the effect of hydrogel composition and imprinting on nanostructure of the hydrogels, ξ , $V_{2,s}$ and $\bar{M}_c$ were calculated. Flory Rehner Equation was used with $V_{2,s}$ which is a measure of amount of the solvent absorbed and retained by the hydrogel to calculate structural parameters such as ξ and $\bar{M}_c$ for hydrogels synthesized without solvent [172]. $V_{2,s}$ was calculated for both loading medium and releasing medium in order to explain loading and releasing behavior of the hydrogels. To performe this, hydrogels which had been loaded in 1 mM DOX solution, were first weighted and then immersed in 0.9 % NaCl solution. After the equilibrium, weights

of the hydrogels were measured. Density of the hydrogels was also measured in dry state. Volume fraction of the hydrogel $V_{2,s}$ is expressed by Equation 4.6 [173].

$$V_{2,s} = \frac{V_p}{V_s} \quad (4.6)$$

where V_p is polymer volume and V_s is the swollen gel volume. If the equation is written explicitly as Equation 4.7 [174]:

$$V_{2,s} = [1 + \frac{\rho_p}{\rho_s} \left(\frac{m_s}{m_0} \right) - \frac{\rho_p}{\rho_s}]^{-1} \quad (4.7)$$

In which ρ_p and ρ_s are polymer and solvent densities, respectively. m_0 and m_s are dry and swollen hydrogel weight, respectively.

The average molecular weight between two crosslink junctions ($\bar{M}_c$) is related with the crosslink degree of the polymer matrix. There are several proposed equation in the literature in order to calculate $\bar{M}_c$ and ξ values which differ with polymerization conditions such as absence or presence of a solvent or cationic, anionic or neutral nature of the hydrogel [175]. For hydrogels prepared in the absence of the solvent, Flory Rehner theory describes the average molecular weight between two crosslink junctions ($\bar{M}_c$) as Equation 4.8 [173,175,176]:

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\left(\frac{v}{V_1} \right) [\ln(1-V_{2,s}) + V_{2,s} + \chi_{12} V_{2,s}^2]}{\sqrt[3]{V_{2,s}} - \frac{V_{2,s}}{2}} \quad (4.8)$$

where:

v - specific volume of the polymer (cm^3/g),

V_1 - molar volume of water ($18 \text{ cm}^3/\text{mol}$),

χ_{12} - parameter of polymer-water interaction

$\bar{M}_n$ - average molecular weight of the polymer chains

If the quantity of $\frac{2}{\bar{M}_n}$ is assumed as negligible ($\bar{M}_c \ll \bar{M}_n$), the Equation for $\frac{1}{\bar{M}_c}$ become

as Equation 4.9.

$$\frac{1}{\bar{M}_c} = - \frac{\left(\frac{v}{V_1} \right) [\ln(1-V_{2,s}) + V_{2,s} + \chi_{12} V_{2,s}^2]}{\sqrt[3]{V_{2,s}} - \frac{V_{2,s}}{2}} \quad (4.9)$$

The mesh size (ξ) is the average correlation distance between two crosslinking junctions. The following equation is used to calculate mesh size of the hydrogels [177]:

$$\xi = \alpha (\bar{r}_0^2)^{1/2} \quad (4.10)$$

α is the elongation ratio of the polymer chains and $(\bar{r}_0^2)^{1/2}$ is the root-mean-squared end-to-end distance of network chains between two neighboring crosslinks in the swollen state. For the isotropic swelling, α is equal to:

$$\alpha = V_{2,s}^{-1/3} \quad (4.11)$$

$(\bar{r}_0^2)^{1/2}$ can be calculated as:

$$(\bar{r}_0^2)^{1/2} = l(C_n N)^{1/2} \quad (4.12)$$

where C_n is the Flory characteristic ratio, l is the length of the bond along the polymer backbone which is 1.54 Å for vinyl polymers, N is the number of links per chain. N is the ratio of $\bar{M}_c$ to M_r and expressed as:

$$N = \frac{\bar{M}_c}{M_r} \quad (4.13)$$

Where, M_r is the molecular weight of repeating units from which the polymer chains are composed of. It is assumed that it is equal to HEMA molecular weight due to small moieties of the other components (HEMA molecular weight= 130.16 g/mol). Combining Equation 4.12 and 4.13, the mesh size of a hydrogel can be easily calculated with Equation 4.14 [178].

$$\xi = V_{2,s}^{-1/3} l \left(C_n \frac{\bar{M}_c}{M_r} \right)^{1/2} \quad (4.14)$$

Solvent interaction parameter (χ_{12}) is calculated with Flor Huggins Theory[179]:

$$\chi_{12} = \frac{1}{2} + \frac{V_{2,s}}{3} \quad (4.15)$$

4.4.10 Cytotoxicity test

The cytotoxicity tests were made at Yeditepe University Tissue Engineering Group. MTT assay was used as methodology to evaluate the effect of molecularly imprinted and non-imprinted hydrogels on the viability of Retinal pigment epithelium (RPE) cells, a cell lines widely used to assess the anticancer activity of a tested compound by virtue of their high stability and proliferation properties. Samples were sterilized

before using in the cell culture. For that purpose, samples were incubated in the ethanol for 3 hours and washed with Phosphate buffered saline (PBS) in 3 times. RPE cell line was used in this study. RPE cells (2.5×10^3 cells/well) were seeded in 24 well culture plate. After 6 hours of incubation for the cell attachment, 1 mL fresh medium and polymers were added into each well and every week 500 μ L of fresh medium containing 10% heat-inactivated fetal bovine serum and 100 units/mL streptomycin was added. Cells were incubated at 37 °C in 5% CO₂ incubator throughout 21 days of incubation. There were 4 groups in the assay: blank (including only medium), only RPE cells (control), only MIP and NIP, and MIP and NIP with RPE cells. At the end of each time period, XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, Cell Signaling Technology) was used according to kit protocol. In this assay, negatively charged tetrazolium salt XTT (in the presence of intermediate electron acceptor PMS) is reduced by viable cells to water-soluble formazan dye. First, the electron coupling (PMS) and XTT labeling reagents were thawed and immediately combined in a 1 μ L : 50 μ L ratio. Then the XTT solution (500 μ L) was added to the cell culture wells. The absorbance at 450 nm was determined after 1, 7, 14 and 21 days of cultivation by using a spectrophotometry (BIO-TEK ELx800 Absorbance microplate reader, USA). Cell proliferation was observed during 21 days.

4.4.11 Drug loading experiments

Both imprinted and non-imprinted hydrogels, were cut into plates using a cork borer of 6.0 mm diameter and were immersed in 100 μ M DOX aqueous solutions (10 ml) and kept at 4 °C for 72 h. All samples were shaken by orbital shaker and protected from light. The samples in the loading solutions were removed after equilibration stage. The amount of the drug that was loaded to hydrogel sample was calculated from the initial and final concentrations of the loading solution after 72 hours. Perkin Elmer Lambda 35 UV/Vis spectroscopy was used in order to measure the drug concentration in the solution. The equilibrium concentration (C_{eq}) value was used to calculate amount of loaded drug as μ g loaded drug/mg polymer. In order to determine amount of loaded drug per gram of hydrogel, the Equation 4.16 was used [137]:

$$Q = \frac{(C_0 - C_{eq})}{m} * V \quad (4.16)$$

where: C_0 , initial concentration; C_{eq} , equilibrium concentration; m , weight of dry hydrogel; V , water volume absorbed by hydrogel.

In order to determine washing solvent efficiency, imprinting factors were calculated and compared for all hydrogels which were washed with different procedures. The imprinting factor (IF) was used as a determine of the strength of interaction between the template and functional monomer for the same compositions. It was calculated according to the Equation 4.17 [70]:

$$IF = \frac{Q_{MIP}}{Q_{NIP}} \quad (4.17)$$

where; Q, loading capacity.

4.4.12 Drug release experiments

In order to observe release kinetics of DOX imprinted and non-imprinted gels, washed and dried discs were firstly placed into 1mM DOX solution for 48 h at 4 °C. After loading, discs were washed with distilled water to remove doxycycline hyclate that was absorbed on the surface and then surfaces of the discs were dried gently by a paper to remove excess water. Each disc was immersed into 10 ml 0.9 % NaCl solution at 37 °C with gentle shaking at 100 rpm in amber bottles. 0.8 ml of releasing solution was withdrawn at regular intervals and the concentration was detected by Perkin Elmer Lambda 25 UV/Vis Spectrophotometer at 274 nm. All releasing performance of hydrogels was carried out in triplicate. After measurement, the outer solutions were immediately returned to the releasing medium. The measurement continued until drug concentration in the releasing medium was stabilized. Drug release kinetic was explained by several models which are present in literature⁴⁻¹¹. Zero-order, Higuchi and Korsmeyer-Peppas Equations were fitted to the release profiles in order to describe releasing transport mechanisms of NIPs and MIPs.

4.4.13 Mathematical models of drug release

4.4.13.1 Zero-Order

Zero order kinetic can be applied to describe the drug dissolution of several types of modified release pharmaceutical tablets, as for constant drug release such as oral osmotic tablets, matrix tablets with low soluble drugs, transdermal systems in coated forms, osmotic systems etc.

Drug dissolution from tablets which do not distributed and released the drug slowly can be expressed by the Equation 4.18 [180]:

$$Q_0 - Q_t = K_0 \times t \quad (4.18)$$

Rearrangement of above Equation gives Equation 4.19:

$$Q_t = Q_0 - K_0 \times t \quad (4.19)$$

where,

Q is the amount of drug released or dissolved, Q_0 is the initial amount of drug in solution and K_0 is the zero order release constant (concentration/time).

To investigate the release kinetics, data collected from drug release studies, are plotted as cumulative amount of drug released versus time.

4.4.13.2 Higuchi kinetic model

Higuchi developed this kinetic model in 1961 in order to define the drug release from matrix system such as, transdermal systems and matrix tablets which have water soluble drugs [181].

Higuchi model was used the following assumptions [182]:

- (i) drug solubility is lower than the initial drug concentration in the matrix
- (ii) one dimensional drug diffusion occurs (edge effect must be negligible)
- (iii) system thickness is higher than size of drug particles
- (iv) matrix swelling and dissolution are negligible
- (v) drug diffusivity is constant
- (vi) the matrix submerge into the release medium perfectly

Higuchi model is expressed by the equation:

$$f_t = Q = A\sqrt{D(2C - 2C_s)C_s t} \quad (4.2)$$

where,

Q is the amount of drug released in time t per unit area A, C is the drug initial concentration, C_s is the drug solubility in the matrix, D is the diffusion coefficient of the drug molecules.

When the drug concentration in the matrix is lower than its solubility, drug release occurs through the pores in the matrix. So, the equation becomes as:

$$f_t = Q = \sqrt{\frac{D\delta}{\tau}}(2C - \delta C_s)C_s t \quad (4.21)$$

where,

δ is the porosity of the matrix, τ is the tortuosity of the matrix. Tortuosity is defined as the dimensions of radius and branching of the pores and canals in the matrix.

After generalization, Higuchi kinetic model can be expressed as [182,183]:

$$f_t = Q = K_H \sqrt{t} \quad (4.22)$$

where,

K_H is the Higuchi constant.

The data collected from releasing studies were plotted as cumulative percentage drug release versus square root of time.

4.4.13.3 Korsmeyer–Peppas kinetic model (the power law model)

Korsmeyer et al. [184] derived an equation which defined drug release from a polymeric system in 1983. To determine the drug release mechanism, first 60 % drug release data were adapted in Korsmeyer-Peppas model. This model is defined by the Equation 4.23:

$$\frac{M_t}{M_\infty} = kt^n \quad (4.23)$$

where,

M_t / M_∞ is fraction of drug released at time t , k is the release rate constant and n is the release exponent which describes the release mechanism of drug for cylindrical matrices as described in Table 4.3:

The Korsmeyer Peppas kinetic model are based on following assumptions [182]:

- (i) The general Equation is practicable for short times and part of the release curve where $M_t / M_\infty < 0.6$ should only be used to calculate the exponent n .
- (ii) One dimensional drug release occurs.
- (iii) The ratio of system's length to thickness should be at least 10.

Table 4.3 : Release exponent values for Korsmeyer-Peppas model [182].

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.45	Fickian diffusion	$t^{-0.45}$
$0.45 < n < 0.89$	Non-Fickian transport	t^{n-1}
0.89	Case II transport	Zero Order release
Higher than 0.89	Super case II transport	t^{n-1}

To study the release kinetics, data attained from drug release studies were plotted as log cumulative drug release versus log time.

4.4.13.4 Drug diffusion coefficient calculations

The diffusion of the drug from a thin film is assumed as one dimensional transport. The ratio of length to thickness was bigger than 10 and edges effect can be neglected. The following partial differential Equation (Equation 4.24) can efficiently define the one-dimensional planar solute release from a hydrogel with initial conditions and boundary conditions by using Fick's law.

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (4.24)$$

$$t = 0 \quad C(x, t) = C_0$$

$$t > 0 \quad x = 0 \quad \frac{\partial C}{\partial x} = 0$$

$$t > 0 \quad x = \pm L \quad C = C_s$$

where, C_0 is initial drug concentration x is distance from center of the sample to the surface, C_s is the surface concentration, C is drug concentration in the hydrogel, D is diffusion coefficient. D is independent from position and concentration and L is the film thickness of the hydrogel in cm.

Equation 4.25 is evaluated with integral transform techniques (Equation 4.25)[185]:

$$\frac{C - C_0}{C_s - C_0} = 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} e^{-\frac{(2n+1)^2 \pi^2 D}{4L^2} t} \cos \frac{(2n+1)\pi x}{2L} \quad (4.25)$$

M_t/M_{∞} represents fractional amount of released drug at infinite time and the Equation is expressed as Equation 4.26:

$$\frac{M_t}{M_{\infty}} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} e^{-\frac{(2n+1)^2 \pi^2 D}{4L^2} t} \quad (4.26)$$

The Equation 4.27 is a complex Equation in order to calculate diffusion coefficient and a solution is found via error functions (Equation 4.27):

$$\frac{M_t}{M_{\infty}} = 4 \left[\frac{Dt}{L^2} \right]^{\frac{1}{2}} \left[\frac{1}{\pi^{\frac{1}{2}}} + 2 \sum_{n=0}^{\infty} (-1)^n \operatorname{erfc} \left(\frac{nL}{2\sqrt{Dt}} \right) \right] \quad (4.27)$$

The integratal complementary error function disappears for short times ($t \rightarrow 0$) and Equation 4.28 is obtained for diffusion [74,100]:

$$\frac{M_t}{M_\infty} = 4 \left[\frac{Dt}{\pi L^2} \right]^{\frac{1}{2}} \quad (4.28)$$

In order to calculate diffusion coefficient, $\frac{M_t}{M_\infty}$ versus $\left[\frac{t}{L^2} \right]^{\frac{1}{2}}$ was plotted and diffusion coefficient is calculated from the slope of the curves.



5. RESULTS AND DISCUSSION

5.1 Optimization of Reaction Conditions

The main factors for designing of imprinted polymer networks are polymerization methods (UV or heat) [44], structure of template molecules, functional monomer and crosslinker, molar ratios of components [58,186,187], reaction temperature [32], and stoichiometry [47]. The formation of a stable complex between template and functional monomer can be achieved with adjusting reaction conditions [44]. At this part of the dissertation, reaction type (UV or heat), initiator concentration, temperature and reaction time have been optimized.

5.1.1 The effect of polymerization method on the reaction rate and monomer conversion

The effect of polymerization method on the reactivity of initiator, conversion and overall reaction rate for imprinted hydrogels was investigated. Our aim is to prepare doxycycline hyclate imprinted hydrogel films for treatment of corneal neovascularization. There are two alternative ways for synthesis imprinted hydrogels; thermal and photo polymerization. Since there are no studies clarifying the effect of template molecules on initiator reactivity, conversion and reaction rate related to the polymerization method, we designed preliminary studies for choosing alternative reaction method. The conversion and reaction rate in photo polymerization reaction of imprinted hydrogels were determined in various doxycycline moieties at constant ratio of functional monomer to crosslinker. The polymerization reaction was initiated with either photo or thermal initiator, and the results were compared. 2-Hydroxy-2-methyl-1-phenyl-propan-1-one is widely used as a photo initiator to initiate acrylates in combination with other monomers (mono or multifunctional) [29,188,189]. After excitation with light, excited initiator created radicals to initiate polymerization reaction. The efficiency of an initiator depends on a number of factors [190]. One factor that influences radical generation is a good association between the absorption spectrum of the initiator and emission spectrum of the light source for high absorptivity [191,192]. The second factor that has important effect on radical generation is

initiator concentration. Studies showed that there is an upper limit for initiator concentration. Above this concentration, the final conversion is not enhanced by increasing initiator concentration. Besides these factors, competitive cases can deactivate radical generation; radical formation is restricted by oxygen, additives or monomer [193,194].

The isothermal reaction kinetic was studied by Fourier transform infrared spectroscopy and differential photo calorimetry. The compositions and assigned codes of the polymers are given in Table 5.1.

Table 5.1 : Formulations used for polymerization in the presence and absence of template molecule.

Code	Initiation type	HEMA (ml)	AA (μl)	EGDMA (μl)	Initiator (mol)	DOX (mol)	DOX:A A
NIP-I-P	Photo	2	14.6	64.3	8.74×10^{-5}	-	-
MIP-1-I-P	Photo	2	14.6	64.3	8.74×10^{-5}	1.33×10^{-5}	1:16
MIP-2-I-P	Photo	2	14.6	64.3	8.74×10^{-5}	2.66×10^{-5}	1:8
NIP-h-P	Photo	2	14.6	64.3	4.23×10^{-4}	-	-
MIP-1-h-P	Photo	2	14.6	64.3	4.23×10^{-4}	1.33×10^{-5}	1:16
MIP-2-h-P	Photo	2	14.6	64.3	4.23×10^{-4}	2.66×10^{-5}	1:8
NIP-I-T	Thermal	2	14.6	64.3	8.74×10^{-5}	-	-
MIP-1-I-T	Thermal	2	14.6	64.3	8.74×10^{-5}	1.33×10^{-5}	1:16
MIP-2-I-T	Thermal	2	14.6	64.3	8.74×10^{-5}	2.66×10^{-5}	1:8

As thermal initiator Vazo 52 was used. Vazo 52 which is known as 2-2'-Azobis(2,4-dimethylvaleronitrile), is a typically used initiator for acrylic and vinyl monomers and has low initiation temperature. DSC measurement was performed to observe the temperature interval that the initiator became active. DSC diagram for Vazo 52 can be seen in Figure 5.1.

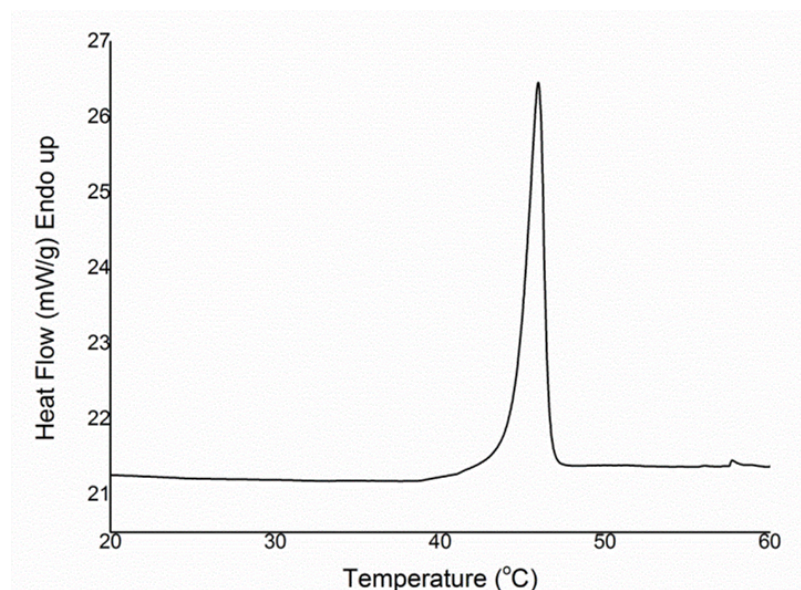


Figure 5.1 : DSC diagram of Vazo 52 initiator.

The height of the peak at about 1635 cm^{-1} decreased with reaction time. The reaction was completed in 10-20 minutes for low initiator concentrations and 2-10 minutes for high initiator concentrations. The FTIR spectra for the synthesis of non-imprinted hydrogels are given in Figure 5.2 as an example.

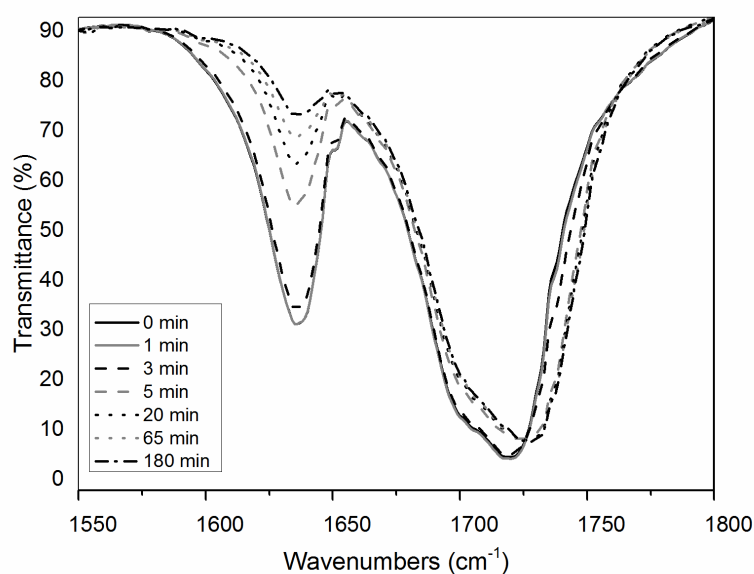


Figure 5.2 : FTIR spectra of the reaction mixture of NIP-I-P coded hydrogel at different time intervals during the photo-polymerization.

FTIR spectroscopy was also used for calculation of conversion of the polymerizations. The conversion, calculated from FTIR data, can be seen in Figures 5.3 and Figure 5.4.

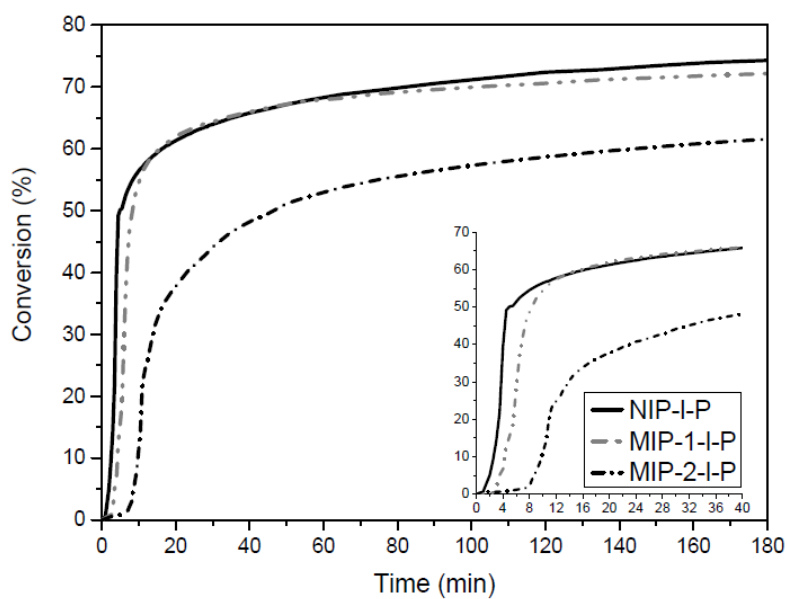


Figure 5.3 : Conversion of double bonds as a function of time from FTIR data for low initiator ratio.

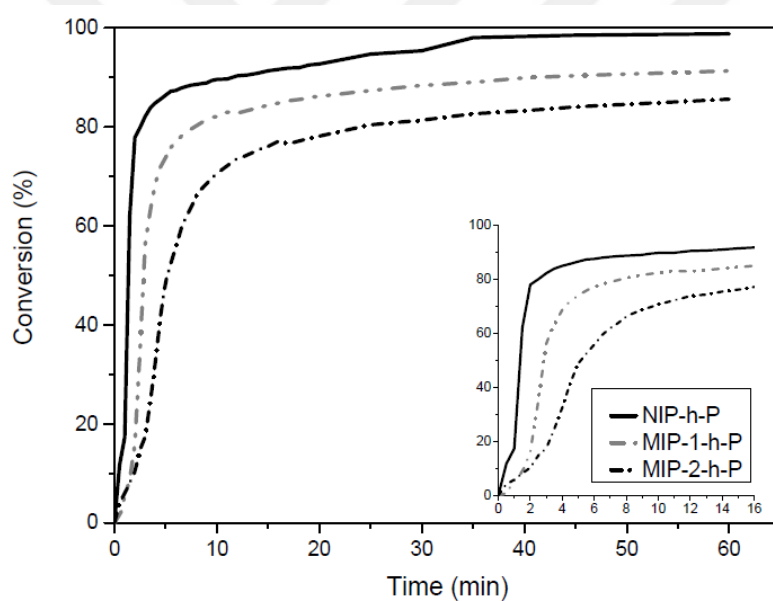


Figure 5.4 : Conversion of double bonds as a function of time from FTIR data for high initiator ratio.

Table 5.2 showed the overall results for conversion of imprinted and non-imprinted hydrogels prepared by photo-polymerization in relatively low (l) and high (h) initiator concentrations at 25 °C.

Table 5.2 : Monomer conversions calculated from FTIR data for UV initiated polymerization.

Code	Conversion (%)			
	UV 1 hr	UV 3 hr	UV+Thermal 16 hr	Thermal
NIP-I-P	68.8	74.3	82.0	-
MIP-1-I-P	68.2	72.1	74.9	-
MIP-2-I-P	53.8	61.5	69.6 (70.1*)	-
NIP-h-P	98.8	-	-	-
MIP-1-h-P	91.3	-	-	-
MIP-2-h-P	85.6	-	-	-
NIP-I-T	-	-	-	86.4
MIP-1-I-T	-	-	-	88.1
MIP-2-I-T	-	-	-	84.5

* After 60 hours (3 hours under UV light + 57 hours in oven)

In general, it is expected that increasing the concentration of initiator in a hydrogel increases the rate of polymerization and the overall monomer conversion. According to the results, the highest conversion was achieved in high initiator concentration in the absence of doxycycline (NIP-h-P). MIP-2-I-P sample which had the highest amount of doxycycline in reaction mixture in the series of low initiator concentration, gave the lowest conversion. The conversion of MIP-1-I-P was slightly lower than that of NIP-I-P, however, the difference was not significant. Even though photo-polymerization was continued for 3 hours at 365 nm and each hydrogel was placed in an oven at 40 °C for 16 hours; the conversion of both MIP-1-I-P and MIP-2-I-P could not be reached the same value as NIP-I-P. To promote conversion for MIP-2-I-P, the reaction mixture was kept also 57 hours at 40°C in oven, nevertheless no higher monomer conversion was obtained. Similar results were obtained for high initiator concentration for UV-initiated system. On the other hand, the conversion was almost the same value for thermal polymerization performed in both the absence and presence of doxycycline. This can be attributed to the fact that DOX influenced the polymerization negatively.

The rate of polymerization reaction was determined by two methods; Fourier transform infrared spectroscopy (FTIR) and differential photo calorimetry (DPC). In Figures 5.5 and 5.6, the polymerization rate (R_p) for each hydrogel synthesized by photo-polymerization, was plotted as a function of time for high and low initiator concentrations.

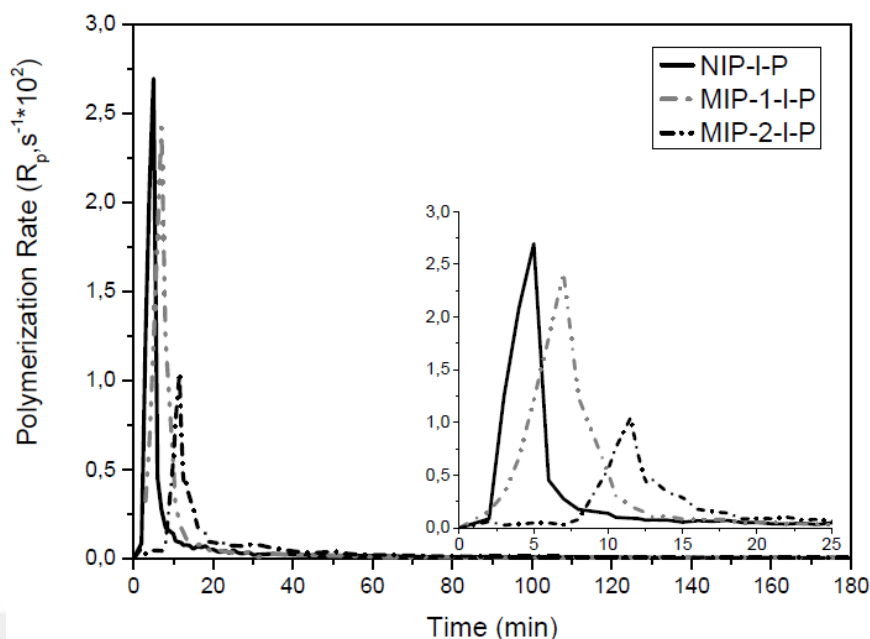


Figure 5.5 : Polymerization rate as a function of time from FTIR data in low initiator ratio for UV initiated polymerization.

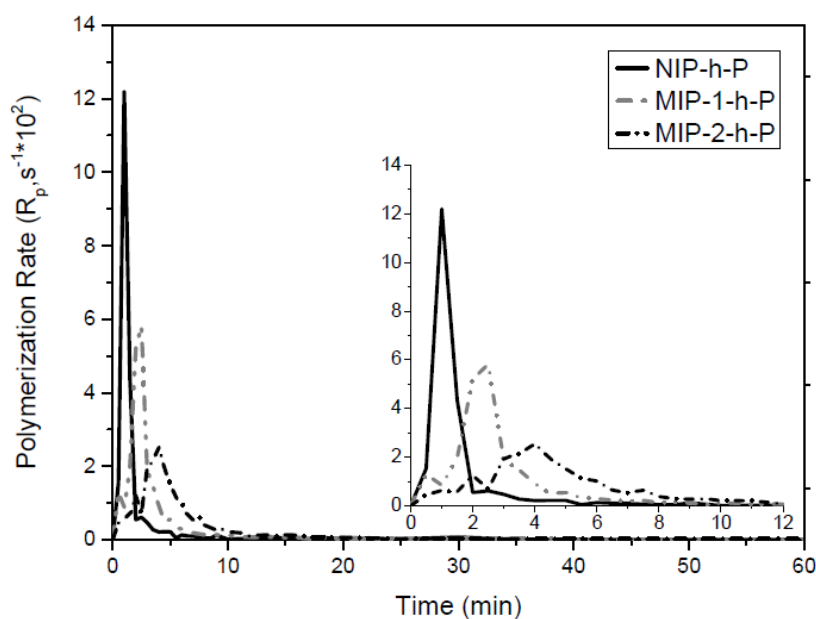


Figure 5.6 : Polymerization rate as a function of time from FTIR data for high initiator ratio for UV initiated polymerization.

The heat flow of the reaction mixture for NIP-1-P, MIP-1-I-P and MIP-2-I-P was obtained by DPC and the polymerization rate was presented in Figure 5.7. The reaction rates of polymerization calculated from both FTIR and DPC data for MIP-coded samples were calculated to be lower than those of NIP-coded samples for each series. Once the amount of DOX increased from $1,33 \times 10^{-5}$ to $2,66 \times 10^{-5}$ mol, the polymerization rate decreased for both high and low initiator concentrations.

Additionally, the induction time shifted to longer time with increasing the amount of DOX in reaction mixture due to higher deactivation effect of the DOX at higher molar ratios (Figures 5.5-5.7).

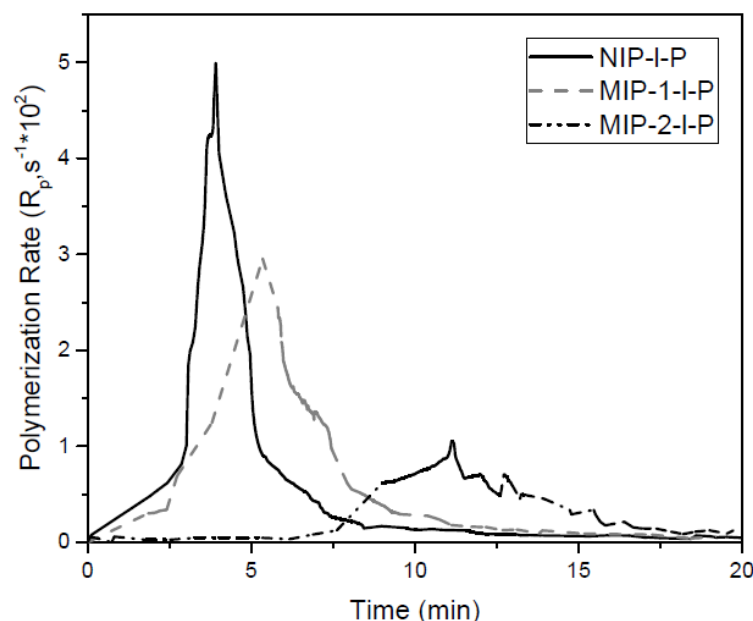


Figure 5.7 : Polymerization rate as a function of time from DPC data for low initiator ratio for UV initiated polymerization.

The obtained results for conversion and overall reaction rate can be explained by chemical structure and sensitivity of the DOX against UV radiation. It can be clearly seen that DOX had a significant influence on the polymerization system. For each specific hydrogel composition, the conversions of imprinted and non-imprinted systems had significantly different values. The effect of the DOX on initiator reactivity can be also seen more specifically from overall reaction rate results. Unexpectedly, the reaction rate and conversion of the imprinted hydrogels have not reached to the non-imprinted ones. In order to provide molecule recognition and enhance imprinting effect, template-monomer interaction in pre-polymerization mixture must be formed. The schematic representation of the reaction was shown in Figure 5.8.

Doxycycline molecule has many functional groups (-OH, -NH, -C=O) capable of making several non-covalent interactions with functional monomer. Acrylic acid monomer provides two functional groups (-C=O and -OH). In the literature, the effect of template-monomer interaction on polymerization reaction has been reported by some researchers [195,196]. These studies indicated that template-monomer interactions caused an initial decrease for imprinted hydrogel polymerization; at the end of the polymerization both conversion and reaction rate had been similar for all hydrogels. So, we realized that

there were some different factors which affected the polymerization reaction in the presence of DOX.

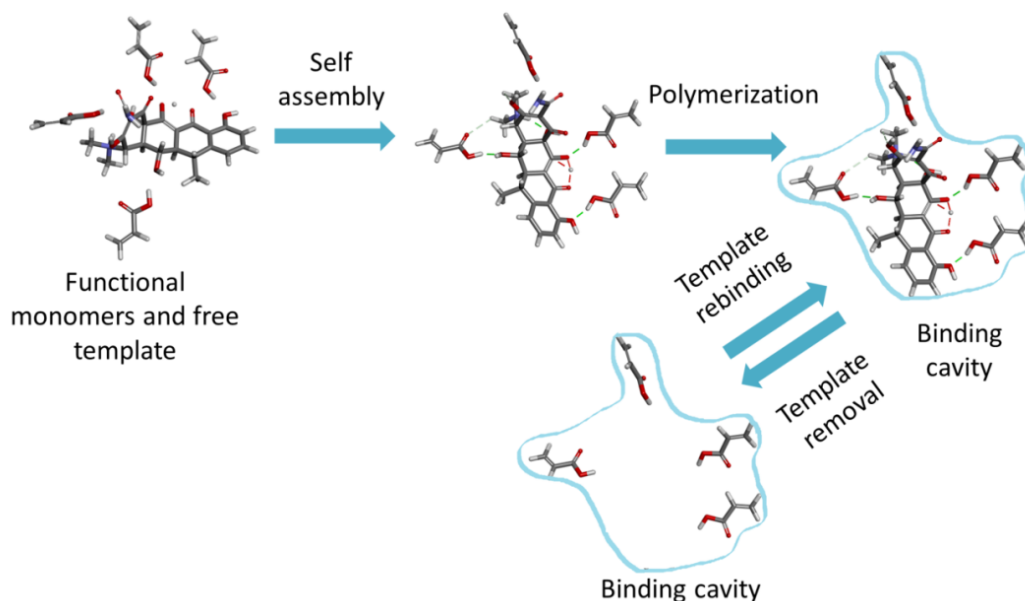


Figure 5.8 : Schematic representation of DOX imprinting procedure.

A reaction pathway was proposed for restriction radical generation in Figure 5.9. DOX absorbed UV radiation at higher wavelength than photo initiator; therefore, the excitation of the initiator could not occur by itself [193]. While initiator had λ_{max} at 322nm, λ_{max} for DOX appeared at 345 nm. However, λ_{max} values for these components were not at the same wavelengths, they were adjacent molecules in the ground state. It was proposed that the DOX was likely to be hindering the radicals generation by the photo initiator and therefore reducing the overall conversion and reaction rate. DOX acted as a UV absorber against to the photo initiator. In the literature, it is indicated that some additives such as pigments in the compositions severely effect the light absorption of the initiator [197,198]. DOX molecules absorbed UV light at higher wavelength than the initiator which blocked the generation of active radicals to form $\text{RM}\cdot$, to decrease the radical generation rate with absorbing UV light. Restricting of the initiator at ground state was unfavorable situation due to the hindrance effect of monomeric radicals, $\text{RM}\cdot$, reducing initiator efficiency. The number of the active radicals produced per photon of light absorbed is restricted by absorbing UV light [198].

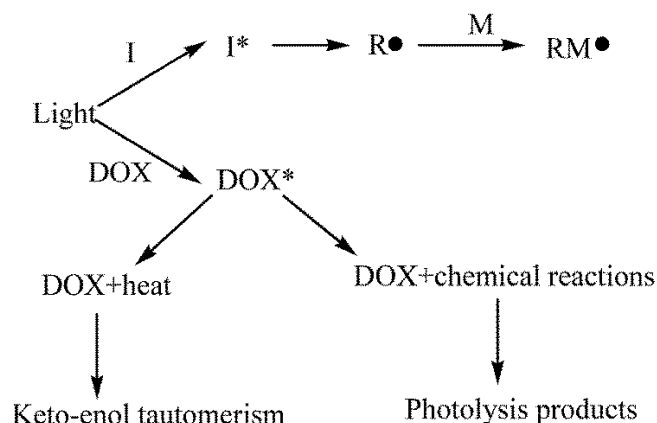


Figure 5.9 : Schematic representation of the possible reaction mechanism for the photoinitiator and DOX structure.

It is explained in the literature that exposure to the light causes to the photo activation of the drug which have chromophore groups (a radiation absorbing substance). Most commonly, wavelengths within the UV-A spectrum (320-400 nm) cause drug induced photosensitivity reactions. Typically, they have at least one resonating double bond or an aromatic ring that can absorb UV energy. Some studies showed that [197,198] photo activation of a chemical occurs with the excitation of electrons from the stable singlet state to an excited triplet state. Excited-state converted into a more stable state with two different pathways for DOX:

1. The excess energy disappeared as heat loss. DOX has a conjugated phenolic diketone group at position 10-12 which caused to keto-enol tautomerization [165,199]. When DOX absorbed the UV light, it was possible to dissipate the absorbed energy as heat via an excited state keto-enol tautomerism like a UV absorber (Figure 5.10) [200]. It is obvious that different tautomeric species may be present in the reaction mixture with different moieties. All tautomers in their different conformations could interact with functional monomer during polymerization. The template-monomer interactions destroyed due to tautomeric conversions.

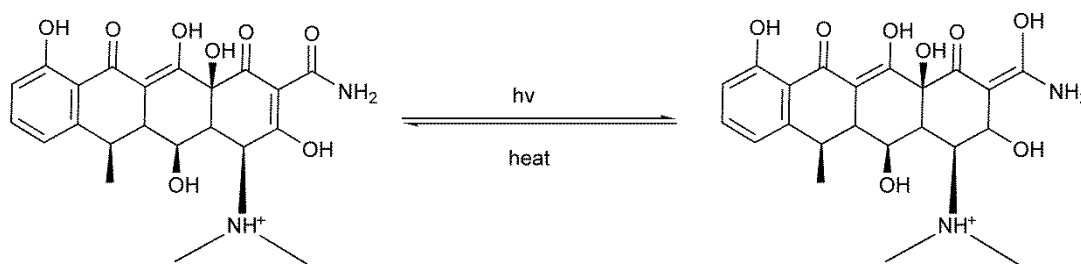
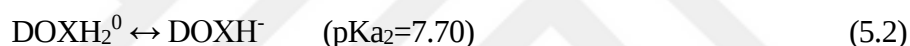
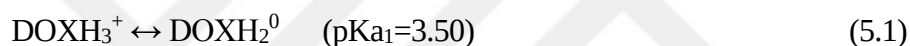


Figure 5.10 : Keto-enol tautomerism of DOX.

2. The chemical reaction(s) may occur depend upon the chemical structure of the molecule. The molecule used the energy of excitation to undergo a chemical reaction. Photolysis is the most substantial process in the degradation of the antibiotic and induced by solar irritation. Degradation with photolysis can be occurred in two ways; direct and indirect. In the direct degradation which is in our case, the UV radiation was absorbed by drug molecule leading to the production of by-products [201]. Jiao et al. indicated that TC^+ formed of TC molecules largely inhibited the photolysis and contrary to this, TC^0 forms encouraged photolysis [202]. Protonation states of the TC give information about the stability of the structure at different pH medium. It was also demonstrated that TC had low stability at high pH values. Negatively charged drug molecules tend to interact with active radicals, therefore strong electrical density of the ring structure causes photo degradation of TCs [202]. In our study the reaction medium was approximately at pH= 3. At this condition, DOX was present in the monomer mixture in $DOXH_3^+$ form, (Equation 5.1-5.3, adapted from ref. [202]).



In the study, DOX molecules may be excited in associated with initiator. In the light of explanations summarized above it is assumed that photolysis reactions occurred during polymerization. It is expected in the study that DOX degradation rate was low even though UV energy absorption occurs during the molecular imprinting photo polymerization at 365 nm.

Finally, we concluded that for the preparation of DOX imprinted hydrogels, thermal induced polymerization is more suitable method than the UV-initiated one.

5.1.2 Determination of initiator concentration, reaction temperature and reaction time

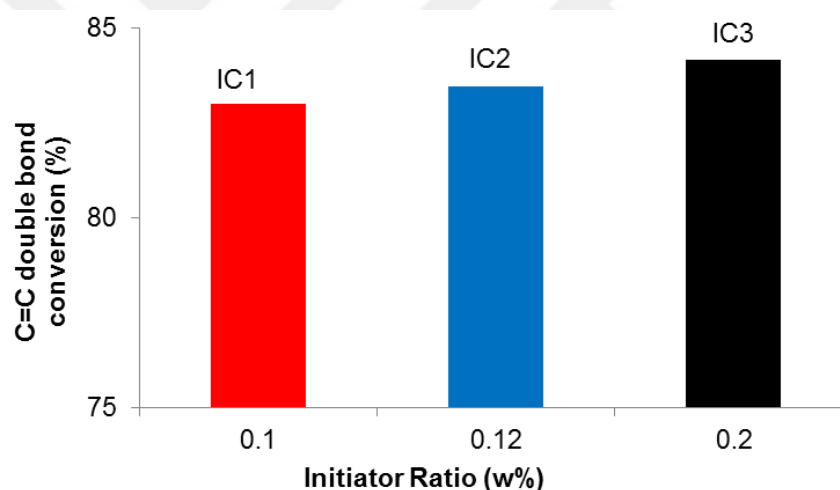
5.1.2.1 Determination of initiator concentration

To determination of initiator concentration, reaction temperature and reaction period, AA and EGDMA were used as functional monomer and crosslinker, respectively. The reaction was performed in three initiator concentrations and formulations are represented in Table 5.3. FTIR spectrums of the reactions are represented in Figure A1.

Table 5.3 : Hydrogel compositions with different initiator concentrations.

Code	Monomer ratio (mol %)	Crosslinker ratio (mol %)	Initiator Concentration (wt %)	Amount of DOX (μ g)
IC1	1.25	2	0.1	2.68
IC2	1.25	2	0.12	2.68
IC3	1.25	2	0.2	2.68

The effect of initiator concentration on C=C double bond conversion is presented in Figure 5.10. IC1 formulated hydrogel was synthesized with 0.0643 mL EGDMA (2 mol %), 0.146 ml AA (1.25 mol %), 2ml HEMA (94 mol %), 2.67 μ g Vazo 52 (0.1 wt%), and 2.68 μ g DOX (1:16 DOX:AA ratio). The Vazo-52 ratio was increased to 0.12 and 0.2 wt% in order to compare initiator concentration effect on the conversion for imprinted polymers. Polymerization mixture was purged with N₂ to remove O₂ for 20 minutes. The polymerization mixtures were then injected into the molds and put into the oven at 50 °C for 36 hours.

**Figure 5.11 :** Monomer conversion in different initiator concentrations.

The increase in the initiator concentration from 0.1 wt % (IC1) to 0.2 wt % (IC3) improved the % C=C double bond conversion from 83.0 to 84.2; this increase was not worth considering (Figure 5.11). When the initiator concentration increased, the kinetic chain length may decrease with an increase in C=C double bond conversion (conversion also depends on termination type and chain transfer possibility) and might attain binding site stabilization. The kinetic chain length is the average number of monomers reacting with a growing chain, and it is inversely proportional to the rate of polymerization and radical concentration [139,203]. Therefore, making attempt to increase polymerization rate by increasing radical species may create smaller sized

polymer chains [139]. So, it has settled in up to 0.12 % wt initiator concentration for imprinted polymerizations.

5.1.2.2 Determination of reaction temperature

The previous studies in the literature indicated that if the thermal initiation is applied for free radical polymerization, the temperature should be as low as possible for a successful imprinting performance [31,204,205]. Keeping temperature at a low value is very important due to the highly dependence of radicalic polymerization kinetics on the reaction temperature. It also affect reaction completion period, conversion and polymer-template interactions. We aimed to achieve adequate monomer conversion at the lowest temperature at this part. Assigned reaction codes and compositions are presented in Table 5.4. FTIR spectrums of the reactions are represented in Figure A2.

Table 5.4 : Hydrogel compositions with different reaction temperatures.

Code	Temp. (°C)	Monomer ratio (mol %)	Crosslinker ratio (mol %)	Initiator Concentration (wt %)	Amount of DOX (µg)
RT1	40	1.25	2	0.12	2.68
RT2	45	1.25	2	0.12	2.68
RT3	50	1.25	2	0.12	2.68

Hydrogels were synthesized with 0.0643 mL EGDMA (2 mol %), 0.146 mL AA (1.25 mol %), 2ml HEMA (94 mol %), 2.67µg Vazo 52 (wt % 0.12), and 2.68 µg DOX (AA:DOX ratio=16). As previously mentioned, polymer mixtures were purged with N₂ to remove O₂ from reaction medium. Subsequently, the polymerization mixtures were injected into the molds and put into the oven at 40, 45 and 50 °C for 36 hours, separately. As it is expected, the reaction conversion increased with increasing reaction temperature (Figure 5.12). The conversion slightly increased from 45 °C to 50 °C and this can be negligible. Increasing the temperature for imprinted polymerization increased overall monomer conversion. It is reported in the literature that increasing reaction temperature may cause loss of the binding affinity of hydrogen bonds and decrease of binding site formation [139,204,206]. Therefore, for non-covalent free radicalic imprinting polymerization the polymer-template interactions are as important as binding site stability. As a result, it has been decided to study at 45 °C for imprinted polymerizations.

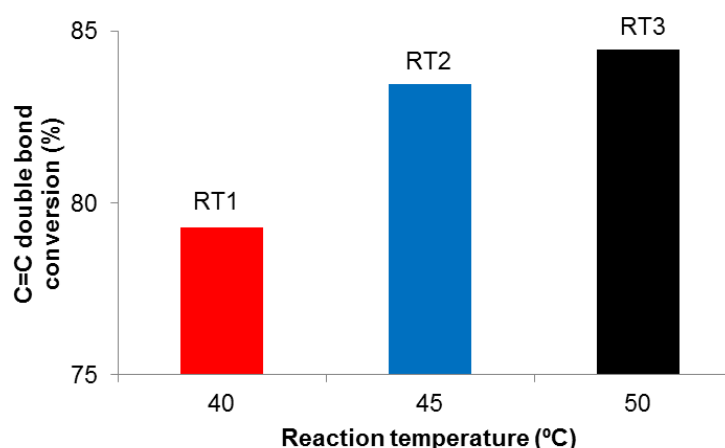


Figure 5.12 : Monomer conversion in different reaction temperatures.

5.1.2.3 Determination of reaction time

Polymerization was allowed to occur for 24-36 hours at a constant initiator concentration (0.12 wt %) and reaction temperature (45 °C). The assigned codes and compositions for the selected reaction time are presented in Table 5.5. It is reported that the reaction time affects the performance (selectivity, loading etc.) of MIPs due to the changes of conversion, polymer rigidity and crosslinker ratio [207].

Table 5.5 : Hydrogel compositions with different selecting reaction temperature.

Code	Temp. (°C)	Monomer ratio (mol %)	Crosslinker ratio (mol %)	Initiator Concentrati on (wt %)	Amount of DOX (µg)	Reac. Time (hour)
RP1	45	1.25	2	0.12	2.68	24
RP2	45	1.25	2	0.12	2.68	36

Once the reaction time extended, the polymerization mixture transformed from liquid to solid form. However, proceeding the reaction time from 24 hours to 36 hours, this case did not increase the conversion significantly (Figure 5.13). FTIR spectrums of the reactions are represented in Figure A3.

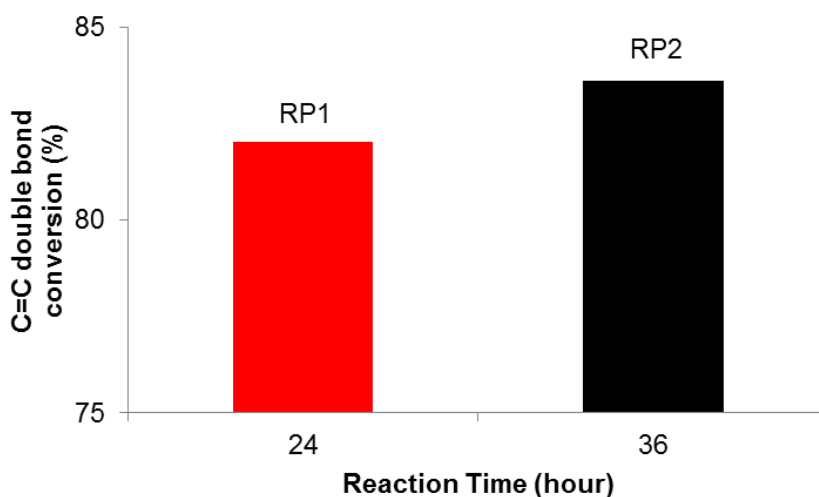


Figure 5.13 : Monomer conversion in different reaction time.

The conversion percentage was calculated as 82.0 and 83.62 for RP1 and RP2, respectively. The conversion slightly increased when the time grew up from 24 h to 36 h. It was considered that temperature treatment of the imprinted hydrogels for a long time, may cause side reactions. Zheng et al. investigated the effect of reaction time and initiator concentration on grafting chitosan and MMA. They offered that increasing reaction time up a certain value may cause side reactions [208]. Consequently, it was decided to keep 24 hours as reaction time for both MIPs and NIPs synthesis.

5.1.3 Optimization of washing solution

The non-covalent interaction of template with the functional groups in the polymer chain was disturbed with applying washing step to eliminate the template, leading to a three-dimensional polymeric structure including cavities matching with the imprinted template [209]. The capacity of MIPs that selectively bind to the target molecule is related to the discharged supplementary cavities. If the washing procedure was not performed precisely in eliminating the template from the crosslinked hydrogel, then the required interaction will not be created to rebind the template molecule [209,210]. Therefore, washing methods must be developed to maximize the elimination of the template from the entire molecular imprinting process.

In this thesis, hydrogels with different compositions were synthesized in order to investigate the effect of washing solution on the hydrogel interaction with the template. The studies were carried out to remove the doxycycline from the polymers by different washing solutions. The effect of the washing solution was determined with four different structured polymers prepared in different functional monomer and

crosslinker combinations. All the MIPs were presented to a washing step, with the aim of disturbing the non-specific interactions between the template and the hydrogel. Both the imprinted and non-imprinted hydrogels were synthesized using the formulations presented in the Table 5.6. After polymerization, both NIPs and MIPs were removed from molds and immersed two times in boiling water for 15 minutes in order to remove unreacted monomers. The disks were cutted in 6 mm in diameter with a corck barrer after removing from boiling water. Then, MIPs were washed to remove template molecule from cavities with different washing solutions at two step.

Table 5.6 : Compositions of the hydrogels used for optimization of washing procedure.

Hydrogel Code	Functional monomer	Functional monomer ratio (mol %)	Crosslinker	Crosslinker ratio	M:T
A	IA	3	TEGDMA	3	16
B	AA	3	TEGDMA	3	16
C	AA	3	EGDMA	2	16
D	IA	3	EGDMA	3	16

The peformance of the washing procedures were determined systematically. Transparent and non-yellowish hydrogels were considered as precisely washed hydrogels. Each experimental part was repeated triplicate. The properties of the solvents which wereused to remove template from MIPs are given in Table 5.7.

Table 5.7 : The properties of the solvents used in washing solutions [107].

Solvent	Polar-Nonpolar	Protic-Aprotic	Dielectric constant	Dipole moment (debye)	Relative polarity
Water	polar	protic	78.5	1.85	1.00
Methanol	polar	protic	32.6	1.70	0.76
Acetic Acid	polar	protic	6.0	1.74	0.65
Acetonitrile	polar	aprotic	37.5	3.92	0.46
Acetone	polar	aprotic	21.0	2.88	0.35

Table 5.8 showed the performance of each washing solution for the first stage and the second stage. The washed and dried hydrogels were immersed in 100 μ M DOX aqueous solutions (10 ml) and kept at 4 °C for 72 h. All samples were shaken by orbital shaker at 100 rpm and protected from light. The samples in the loading solutions were removed after equilibration stage. Then, Perkin Elmer Lambda 25 UV/Vis Spectrophotometer was used to measure the initial and final absorbance at 274 nm. The results for each case were determined by calculating the imprinting factor (IF) and

compared with each other in order to determine the efficiency of the wash solution. The unloaded but washed hydrogels were immersed in releasing medium in order to determine washing completion. We also found that the template could not longer be detected in releasing medium with UV spectrophotometer at 274 nm.

Eliminating methanol from first step washing mixtures which was the most polar solvent after water (Mixture 4) caused to imprecise washing. The hydrogels were not cleaned and the template was not passed to washing solution. Due to oxalic acid:acetonitrile (Mixture 4) was not succesful to remove template from the MIP because the color of the hydrogels were yellowish after first step washing. The second step washing procedure was not applied to Solvent 4.

Table 5.8 : Solvent mixtures and observations.

Number of Solvent mixture	Mixture	Mixture Ratio (volume)	Observations after first washing step	Second step of washing	Observations after second washing step
1	Oxalic acid solution : methanol:acetonitrile	65:15:20	The template was partially removed from hydrogel. The color of the hydrogel was light yellow.	Methanol Acetonitrile	Transparent Light yellow. The hydrogels discs adhered to glass plate after drying step.
				Acetone	Light yellow
2	Oxalic acid solution: methanol	65:20	The template was partially removed from hydrogel. The color of the hydrogel was light yellow.	Methanol Acetonitrile	Transparent Light yellow. The hydrogels adhered to glass plate after drying step.
				Acetone	Light yellow
3	Acetic acid:methanol	1:9	The template was partially removed from hydrogel. The color of the hydrogel was light yellow.	Methanol Acetonitrile Acetone	Transparent Transparent Light yellow
				-	The second step was not applied to hydrogels due to unsuccessful first step.
4	Oxalic acid solution:acetonitrile	65:15	The template was not removed from hydrogel effectively at this step. The color of the hydrogel was yellow.	-	

Loading capacities (Q , $\mu\text{g DOX/mg dry hydrogel}$) and min-max values of IF were calculated for both NIPs and MIPs and obtained results were given in Table 5.10. It has been aimed to use the washing procedure in which the imprinting factor is relatively high for hydrogels in the upcoming stages of the experimental studies. The lowest IF values were obtained for all synthesis which were washed with acetone in the second step. Acetone, as an aprotic solvent, has the lowest polarity of the solvents used in this study. It has been observed that, after washing with oxalic acid solution: methanol solvent, the IF values of hydrogels which were washed by acetonitrile in the second stage were higher than the other second step solvents. However, when the cleaning performances of the washing solutions were compared, it is seen that even if the IF value was high, the films were not cleaned after the second stage washing. The photographs of C-coded hydrogel were given as an example in Table 5.9.

Table 5.9 : Appereance of the hydrogels after washing with different procedures for sample C.

Number of Mixture Solvent	Sample C		
	Methanol	Acetonitrile	Acetone
1			
2			
3			

The photographs were obtained after drying of washed hydrogels. C-coded hydrogels which were washed with Mixtures 1 and 2 and were washed with acetonitrile in the second stage, protected their yellow color displaying that the drug is not completely cleaned. Methanol in the second stage in combination with solution 1 (oxalic acid solution: methanol: acetonitrile), which had a higher IF and better cleaning performance than the others, was decided to use as washing procedure for MIPs.

Some studies in the literature indicated that high polar solvents should be used in the washing solutions to reveal the difference between the specific interaction and the non-

specific interaction between the hydrogel matrix and template . It is worthy to note that methanol was a protic solvent with the second highest polarity after water.

ANOVA ($p < 0.05$) was also used to investigate the effect of first step solvent mixture type, hydrogel composition and second step solvent type on the loading capacity and IF values of samples. Statistically, the loading capacity of the samples (have different monomers and crosslinkers) improved with Mixture 1. Mixture 2 and 3 caused a decrease in IF values leading to deterioration of specific interactions. According to the statistical analysis, comparing the cleaning performances of the washing solutions indicated that when the IF was high value, the films were not cleaned in some samples (Figure 5.14). Therefore, it was decided to use the washing steps combination that eliminate template from hydrogel as well as provided relatively higher IF value during the studies and removed effectively template from the hydrogel. Methanol was thus able to weaken the non-specific interactions occurring between the template and the hydrogel, moreover, it helped retain the specific interactions between the template and the binding sites in the MIPs. In this way, more effective imprinting could be achieved. The results showed that B coded sample had the lowest IF values after all washing procedures. This was related to its chemical structure not the washing procedure.

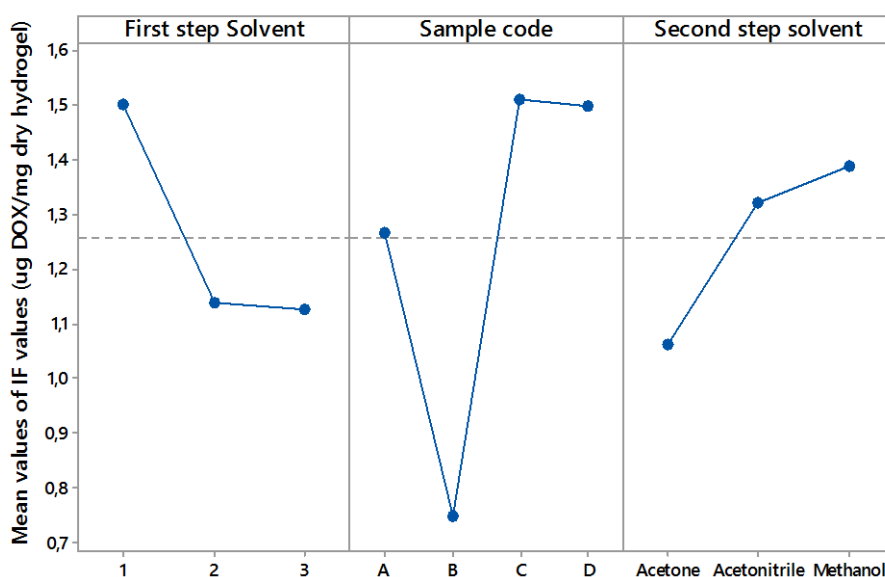


Figure 5.14 : Effect of solvents on imprinting factor of the hydrogels.

It has been proved that the condition selected for washing the MIP play essential role in loading capacity of the MIPs. Therefore, oxalic acid: methanol:acetonitrile was

selected as the washing solvent in the first step, and methanol was selected as the suitable second step solvent.



Table 5.10 : The dataset obtained from the experimental results.

Hydrogel Code Mixture		Acetone			Acetonitrile			Methanol		
		Q _{MIP}	Q _{NIP}	IF	Q _{MIP}	Q _{NIP}	IF	Q _{MIP}	Q _{NIP}	IF
A	1	2.20-2.38	1.86-1.96	1.12-1.23	1.42-1.49	1.86-1.96	1.43-1.56	1.76-2.02	1.86-1.96	1.23-1.30
	2	3.04-3.22		1.55-1.73	3.17-3.21		1.61-1.70	2.31-2.54		1.24-1.30
	3	1.31-1.96		0.63-0.84	1.74-2.01		1.02-1.08	1.96-2.27		1.05-1.16
B	1	0.85-1.24	1.57-1.77	0.54-0.70	0.99-1.42	1.57-1.77	0.63-0.80	0.8-1.28	1.57-1.77	0.51-0.72
	2	1.53-2.05		0.97-1.16	1.82-2.34		1.16-1.32	1.44-1.92		0.92-1.08
	3	0.92-1.39		0.61-0.78	0.77-0.98		0.49-0.55	0.33-0.66		0.21-0.37
C	1	0.63-1.18	0.42-0.52	1.50-2.26	0.90-1.47	0.42-0.52	2.14-2.83	0.74-1.33	0.42-0.52	1.77-2.56
	2	0.13-0.19		0.30-0.36	0.28-0.65		0.66-1.24	0.47-0.80		1.12-1.53
	3	0.36-0.61		0.9-1.17	0.44-0.76		1.04-1.45	0.8-1.29		1.9-2.48
D	1	1.28-1.78	0.89-1.01	1.43-1.76	1.55-2.22	0.89-1.01	1.74-2.20	1.65-2.22	0.89-1.01	1.85-2.20
	2	0.45-0.85		0.50-0.84	0.63-1.22		0.71-1.21	1.26-1.79		1.42-1.77
	3	1.05-1.45		1.18-1.44	1.26-1.72		1.41-1.70	1.44-1.04		1.62-1.99

5.2 Investigation of the Interaction of Template with Monomers and Crosslinkers

5.2.1 Characterization of complex based on the template interaction

Absorbance spectroscopic titration [71,211,212] study was one of the method which was carried out to quantify the complexation of the functional monomers and crosslinkers with the template, DOX. In this section, we defined UV spectroscopic analysis of the molecular imprinting pre-polymerization step, where interactions between the template-functional monomers and template-crosslinkers were subject to thermodynamic control [212]. The titration of functional monomers and crosslinkers with doxycycline hyclate, in ethanol with corresponding ratios used in pre-polymerization mixture, was monitored by UV-Vis spectroscopy. The observed shift in both $\lambda_{\max}$ and absorbance values were used to estimate the degree of complex formation.

Our results indicated that (i) The band intensity increased for all monomers and crosslinkers with different degrees according to their structures and there was a direct function of monomer:template and crosslinker:template ratio (Figure 5.15) Once the template ratio was remained constant and the monomer was increased, the absorbance shifted to higher value (X: T and M: T increased) (Figure 5.16).

DOX molecules were predominantly in cationic form in ethanol. The binding of DOX to crosslinkers and monomers was more effective through hydrogen bonding and electrostatic, which caused the absorption peak of the pre-polymerization solutions to shift to blue. Any complexation may lead to shift to a lower or higher wavelength with higher absorbance due to the structure of the bond. In the case of hydrogen bonding, which was a relatively weak interaction, the shift was to lower value. Depending on the nature of the monomer or crosslinker, the complexes were differently influenced by the template in terms of interaction strength. Although titration of IA with DOX solutions in ethanol provided noticeable absorbance changes after equilibration, titration of AA into ethanol solutions showed quite small shift in the absorption spectra, indicating that the AA as a functional monomer less interacted with the template than IA (Figure 5.15). Itaconic acid is a weak acid and unlike monocarboxylic acrylic acid, it has two carbonyl and two hydroxyl groups on its structure, which was capable of making strong hydrogen bonds with the corresponding groups [213]. IA

might interact with doxycycline molecule strongly, consequently, it was a strong candidate as a functional monomer in imprinted polymers which bind the doxycycline via non-covalently. Therefore, the identification by absorbance titration of itaconic acid as a high affinity monomer for doxycycline hyclate has been presented to be an effective method in designing molecular imprinted polymers that can be used for controlled drug release.

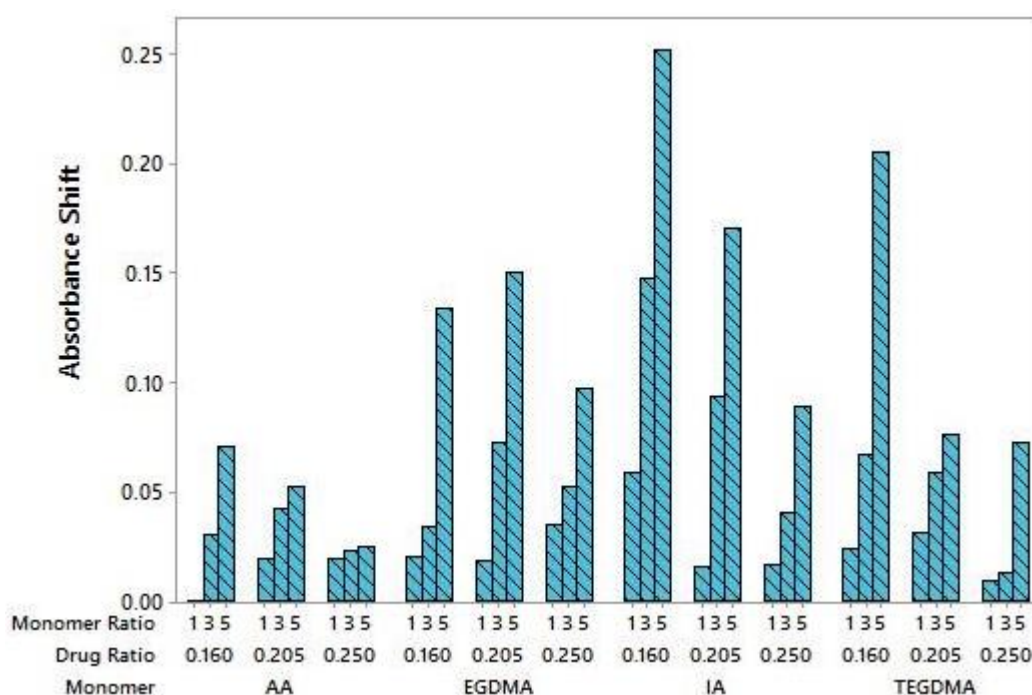


Figure 5.15 : UV absorbance changes due to complexation between template and other polymerization components.

Interaction of TEGDMA and EGDMA with DOX in ethanol provided obvious absorbance changes at high template ratio (0.205 and 0.250 mol %) in all drug ratios. EGDMA and TEGDMA were polar molecules and interacted with DOX according to the results. On the contrary, if the absorbance changes were compared with each other, it could be seen that interaction of TEGDMA with doxycycline hyclate was less than that of EGDMA at high ratio (5 mol %). It may be interpreted that TEGDMA could not provide the expected interaction with the template. We can assume that ether groups of TEGDMA may not offer the same potential for interaction with template. Shoravi et al. proved computationally that crosslinker with higher number of functional group did not interact with template via all groups [18]. Andersson et al. demonstrated that there was no significant change in the absorption spectrum and therefore there was no interaction between EGDMA and the template molecule [212]. The interaction of crosslinker with template plays important role in controlling the

number of non-specific binding sites. In order to decrease the non-specific binding sites, which is present in the polymer structure after polymerization, crosslinker-template interaction should be small or insignificant compared with the relevant monomer [212].

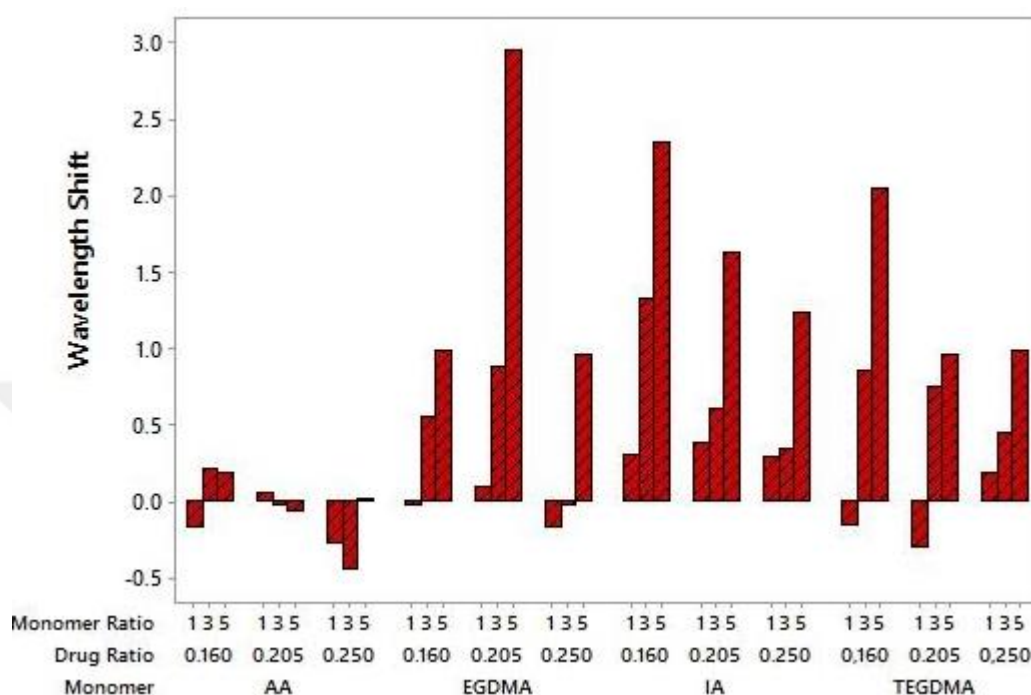


Figure 5.16 : Wavelength shifts due to complexation between template and other polymerization components.

Hydrogen bonding played a vital role on UV absorption alterations. Doxycycline structure carries various functional groups on its structure that made it possible to form both intramolecular and intermolecular interactions, especially strong hydrogen bonding. Figure 3.5 demonstrated hydrogen bond positions of doxycycline which generate intramolecular interaction with the neighboring functional groups. This caused in decrease in the capability of the hydroxyl group in making hydrogen bonds with other molecules.

We assumed that, once the hydroxyl group acted as a proton donor, a red shift (shift to higher wavelength) was observed and acting as a proton acceptor, as seen here, a blue shift (shift to shorter wavelength) appeared (Figure 5.16).

Functional groups such as O-H from monomers or crosslinkers were involved in intermolecular hydrogen bonding in the presence of proton acceptors i.e. oxygen and nitrogen from doxycycline hyclate. As the DOX concentration increased, intermolecular hydrogen bonding decreased. Additionally, changing monomer or

crosslinker concentration affected intensity of the intramolecular hydrogen bonding with increasing absorbance value. Therefore, hydrogen bonding shifted the λ_{max} to the shorter wavelength for this system(except AA). Small blue shift for the monomers and crosslinkers was due to the C=O groups in O-H $\cdots$ O hydrogen bonding. For molecules having a hydroxyl group, the direction of the spectrum shifts induced by the hydrogen bond formation depends on whether the molecule acts as a proton donor or acceptor which were obtained by Hu et al. They indicated that l-dopa molecules interacted with MIP films and its UV spectrums shifted to low wavelength because of intramolecular interactions [214]. In another study, of CuCl₂ and 4-vinyl pyridin (4-VP) complexation was investigated by Zhang et al.[211]. Their results showed that the absorbance increased and the wavelength shifted to lower values with increasing CuCl₂ concentration in 4-VP solution.

In brief, both crosslinkers and monomers which was used to prepare DOX-imprinted hydrogels were interacted with DOX in different interaction levels from different binding regions according to thir natures and ratios.

5.2.2 Investigation of the crosslinker template interaction with binding studies

The crosslinker forms the skeleton of the polymer network. It should fix the cavities stable and protect its shape after washing process. One of the methods was used to select the crosslinker comparison of the template binding scores of the different crosslinkers [215,216]. Within the scope of the thesis, affinity binding assay was applied to EGDMA and TEGDMA to identify affinity of the crosslinkers to the doxycycline. Consequently, TEGDMA and EGDMA polymerized with thermal initiator at 45 °C and p(TEGDMA) and p(EGDMA) samples were obtained. Then, they were loaded with doxycycline for 72 hours at 4 °C with shaking gently at 100 rpm. The loading results were shown in Figure 5.17. As it can be seen, the binding capacity of DOX varied in the order TEGDMA>EGDMA.

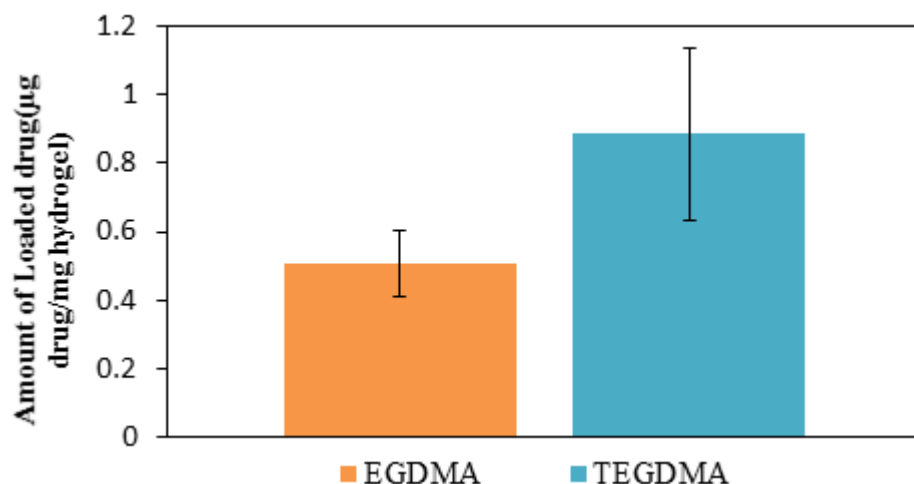


Figure 5.17 : Amount of loaded drug by crosslinkers.

It might be due to the fact that the mesh size of p(TEGDMA) was sufficient to keep the drug molecules stable in the matrix and TEGDMA film absorbed more drug than EGDMA film. On the other hand, according to absorbance titration results, the template molecule-monomer and template molecule-crosslinker interaction varied as a function of their ratios. For these reasons, we decided that long and short chain crosslinkers should be compared with the studies in order to use large and small size monomer. The effect of the monomer template combination on loading and releasing performances of the hydrogels could be investigated by selecting crosslinkers with different functional group numbers or chain lengths.

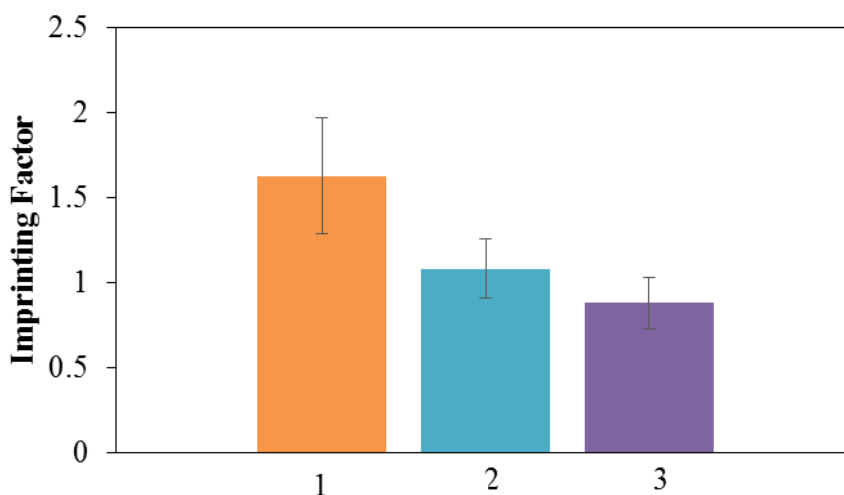
5.2.3 The effect of functional monomer on the imprinting process

A main factor in molecular imprinting is the ability of functional groups to interact with the template molecule to achieve a certain degree of order alignment and specialized interactions (hydrogen bonding, Van der Waals, ionic interactions *etc*). At this section, the synthesis of the imprinted polymers were carried out to investigate the effect of the functional monomer material on the imprinting results. Hydrogels were synthesized with TEGDMA and two different functional monomers (IA and AA) using a fixed initial M:T ratio of 18. The mol ratios of the components in the hydrogels were given in Table 5.11. The crosslinker: monomer ratio was chosen to be 1:1.

Table 5.11 : Compositions of the hydrogels and monomer ratio in binding DOX.

Hydrogel Code	Functional Monomer	F.monome r ratio (mol %)	Crosslink er	Crosslinke r Ratio (mol %)	HEMA Ratio (mol %)
1	IA	1	TEGDMA	1	98
2	AA	1	TEGDMA	1	98
3	-	-	TEGDMA	1	99

The Figure 5.18 showed the binding efficiency for MIPs and NIPs in terms of IF. It is clear that MIP synthesized with IA, exhibited the highest imprinting factor. Hydrogel with no functional monomer had a lower imprinting factor. Depending on the 1mol % TEGDMA ratio used in the synthesis of hydrogels, IA>AA>HEMA provided effective imprinting properties to hydrogels, respectively. On the other hand, the DOX-MIP synthesized with IA and TEGDMA were not only ‘recognized’ DOX, due to the presence of the functional monomer, but shape complementarity may also be responsible for the loading in the DOX-MIP. Altogether these results showed that using DOX in the pre-polymerization mixture was successful in arranging the monomers and thereby forming a hydrogels with a surface architecture optimized for the recognition of the template. A comparable study were made by Lin et al. and they were selected maximum selective functional monomer which had higher imprinting factor [98].

**Figure 5.18 :** The imprinting factors for imprinted polymers which is synthesize with IA, AA and HEMA as functional monomer.

5.3 Formulation, Synthesis and Evaluation of Doxycycline Hyclate Imprinted Hydrogels for Controlled Drug Delivery

The objective of this section is to optimize the formulation of DOX- imprinted hydrogels using fractional factorial design. Both non-imprinted and imprinted hydrogels were synthesized by thermal radicalic polymerization. Five factors including monomer type, monomer ratio, crosslinker type, crosslinker ratio and template ratio have effect on the loading and releasing of drug from imprinted hydrogels. Although crosslinker is considered as an important feature, there is a little research on how the template-crosslinker interaction contributes to the recognition properties of the final material [167,217]. EGDMA is the most known and used crosslinker for MIP synthesis. TEGDMA which has a long flexible glycol chain crosslinker, was also used to prepare a variety of resins and MIP membranes and contact lenses [49]. The crosslinker constituted equal or high amount comparing with functional monomer of the MIP by mole (in this work 1–5 mol %); therefore, it ordered the arrangement and rigidity of the polymer network, and potentially contributed to a noteworthy amount of the non-specific binding [49]. Crosslinker ratios were selected from 1 to 5 mol %. It was believed that ratios equal to or greater than 5 produced a MIP lacking flexibility to allow mass transfer to the binding site of the template molecule rapidly, whereas conversely, a MIP formed at 1 % or less molar had not have enough mechanical strength to maintain the binding site memory and increased undesired interaction with template. Values for the monomer were selected between 1 and 5 mol % ratio. Using ratios equal to or lower than 1 can produce binding sites with too little occasion for template interaction. On the contrary, using ratios above 5 can cause to create binding sites with too many points for template interaction and also increase unspecific interaction which can then not be specific for template [218]. Monomer and crosslinker quantities were determined with reference to imprinted polymer synthesis made without solvent [21,33,172,185].

In the development of extended formulations with various factors, it is difficult to determine factors influencing drug release behavior of the hydrogels. The success of the imprinting method depends on the appropriate selection of the chemical factors that affect the properties of the synthesized polymers from their structure to morphology. However, both the morphology of the polymer and the chemical organization of the binding site are important to the whole performance of the MIP,

the number of experimental parameters and their potential interactions which may effect organization of binding sites and morphological properties of the MIPs. Therefore, imprinted polymers are proper samples for combinatorial synthesis, permitting the main factors to be quickly screened [89].

The experimental design and the statistically data analysis are two features of any experimental problem that should be taken into condiseration. These two subjects are closely related to each other because the analysis method is affected by the design used for experiment. The statistical design of experiments involves the process of planning the experiments for the collection of appropriate data that can be analyzed by statistical methods, resulting in valid and objective results. The most important objective is to create representative and informative experiments with a minimum number of experiments. Since two or more factors are investigated, selection of the full factorial design was very well-organized. But, there are some drawbacks of the full factorial design. Five factors is designed experimentally as 32 (2^5) runs with full factorial method. We can evaluate 5 main effects, 10 two-factor interactions, 10 three-factor interactions, 5 four-factor interactions, and 1 five-factor interaction. 16 of them are used to investigate three-factor or higher order interactions. Though, it is established that three or higher order interactions can be negligible in the literature [168]. It can be accepted that the half of the interactions are hypothetically ineffective. Hence, it is a pointless choice to study five variables in 32 runs without center points and non-imprinted conjugates. Fractional factorial design is more practical method for evaluation of lower order interactions. The model displays which factors are important, how they are combined to influence the process, identify the patterns in the data, and suggest the predicted optimums with minimum number of experiments [89,219–222].

A fractional factorial design based on a fraction of the full factorial ($1/2$, $1/4$, $1/8$, ..., $1 / 2^p$) applies where the various variables need to be investigated. The number of experiments includes 2^{K-p} experiments where p is the size of the fraction. Therefore, the total number of experiments in the fractional factorial design is much less than the full factorials design method. For this reason, this multi variable optimization method was used in this study. As shown in Table 4.2, five variables were investigated, but the initiator concentration, initiation type, reaction time and reaction temperature were fixed with preliminary studies.

5.3.1 Statistical analysis and modelling studies

This section defined a method for preparing and screening a library of various imprinted hydrogels through the application of an experimental design and the use of a multivariate analysis methodology to improve selected key factors in the preparation of high affinity MIPs. Five factors that have a significant effect on the drug loading/releasing performance of MIP were investigated using a two-level fractional factorial design. The ratio and type of functional monomer, the ratio and type of crosslinker and the ratio of template were selected as independent variables and Time for 90 % of Drug Released (Y1), Amount of Drug Released in 4 h (%) (Y2), Total Amount of Released Drug in 48 h (Y3) and n_1 values obtained from Korsmeyer Peppas Equation (Y4) were identified as dependent variables (i.e., responses) (Table 4.2). The drug release performance was directly affected by composition of the hydrogel. Ideal imprinted hydrogels are proposed to possess the following characters: (1) biocompatible; (2) high imprinting effect; (3) controlled delivery for DOX. Thus, in the study we decided to gain an insight to the binding properties, the releasing properties, and cell viability. The effect of independent factors and their interactions on the dependent variable were investigated. Firstly, the effect of variables in the molecular imprinting system was considered by using a $2^{(5-1)}$ fractional factorial design carried out with center points. Essentially, each fourth-order interaction aliases with the appropriate main factors: $A=BCDE$, $B=ACDE$, $C=ABDE$, $D=ABCE$ and $E=ABCD$. And also, each two order interaction was aliased with a third-order interaction; for instance: $AB=CDE$, $AC=BDE$, ..., $DE =ABC$. To remove these effects, it was assumed that high-level interactions were negligible because a common and logical assumption was that high-level interactions were less important than low-level interactions [168]. Hence, we can consider all the main factors and all two- order factorial interactions, assuming that the third and higher interactions are insignificant, which was logical in practice. Effect aliasing was a result of using fractional factorial design.

Although the wide-scale study in molecular imprinting, there are many factors that affect the properties of MIPs, and there are no well-developed rules to follow in designing target binding cavities on the polymer that exhibit the desired recognition properties. This can be due to the uncertain understanding of the interactively effects between these factors. The complexity of the imprinting process and the use of a

combinatorial approach through the application of an experimental design allows the factors to be screened to assess its effect on the properties of the MIPs and thus optimize the polymer composition [89].

The amount of release DOX of each formulation was measured using UV spectrophotometer to obtain a multivariate model that identifies the factors that determine the recognition features of the MIPs. The hydrogels were prepared as a thin layer in the glass molds using two glass plates separated by a 0.45 mm wide silicone frame following the thermal polymerization method as mentioned previously. The MIP imprinting was compared with corresponding control polymers (NIPs), which synthesized following the same protocol without the using of doxycycline hyclate.

Table 5.12 : Hydrogel compositions of the DOX-MIP library according to a 2^{n-1} fractional factorial design of four center points.

Hydrogel Code	Center Point	A	B	C	D	E
1	1	AA	1	EGDMA	1	0.250
2	1	AA	5	EGDMA	1	0.160
3	1	AA	1	EGDMA	5	0.160
4	1	AA	5	EGDMA	5	0.250
5	0	AA	3	EGDMA	3	0.205
6	1	IA	1	EGDMA	1	0.160
7	1	IA	1	EGDMA	5	0.250
8	1	IA	5	EGDMA	1	0.250
9	0	IA	3	EGDMA	3	0.205
10	1	IA	5	EGDMA	5	0.160
11	1	AA	1	TEGDMA	1	0.160
12	1	AA	5	TEGDMA	1	0.250
13	1	AA	5	TEGDMA	5	0.160
14	1	AA	1	TEGDMA	5	0.250
15	0	AA	3	TEGDMA	3	0.205
16	1	IA	1	TEGDMA	1	0.250
17	1	IA	5	TEGDMA	5	0.250
18	1	IA	1	TEGDMA	5	0.160
19	1	IA	5	TEGDMA	1	0.160
20	0	IA	3	TEGDMA	3	0.205

Table 5.12 showed the setting of optimization design chosen for each factor in this study. These settings were chosen by considering the literature review about solvent free polymer imprinting process with different templates which have various number of binding sites.

The numbers of synthesized polymers for each MIP library was 20 (2^{5-1} plus 4 center points) and 20 for the non-imprinted polymer libraries according to the settings. For

preparing of non-imprinted polymers, polymerization mixtures were prepared without doxycycline hyclate. For each polymer library, the samples were used as a model set to obtain empirical multivariate models. The ratio of M:T was considered to be the response in the multivariate analysis. The experimental values of the responses (OV) were quantitatively compared with the predicted values (FITS) to calculate the prediction bias (Residual). Compatibility and predictability of the models were assessed by the FITS and Residuals values estimated from regression equations. It should be mentioned that the MIP and NIP libraries were modelled together.

An observation of each calibration model was carried out by utilizing the regression coefficients of the model and the sum of square values [61]. In the coefficients diagrams, the relative magnitudes and signs of the coefficients related to the center and scaled factors exhibited the effect of each model term, and reflected the underlying structure of the data [61]. Additionally, the sum of square values of each factor which had p value less than 0.05 provided a shortened summary of the importance of the factors with adherence to the response variables, permitted sorting all factors reducing importance in fitting the model. Moreover, some factors had p value higher than 0.05. In such cases, they were removed from the model as outlier and the model was refitted. Then, stepwise regression was performed and confirmed that there was no other significant effect having p value lower than 0.05.

5.3.1.1 Y1: Time for 90 % of drug released

Time for 90 % drug released provided information about biphasic system including both two phases (relatively fast and slow phases). The model calculated from polymer library was slightly good and exhibited 64.12 % prediction ability of the response variance (Table 5.13), and the model showed a strong and quantitative interaction between the two factors which estimated the time for 90 % of drug released from the samples (Table 5.14).

Table 5.13 : Model summary for response Y1.

R-sq (%)	R-sq (adj) (%)	R-sq (pred) (%)
67.8	64.12	58.28

The FITS and Residual values were presented in Table 5.15. Residual analysis revealed that the ordinary assumptions on the error were well-advised. The main and interactively effects on the Y1 response were presented in Figure 5.19 (a-b) and the

most influential factors on the models were presented in Table 5.14 for the polymer libraries for both MIPs and NIPs.

Table 5.14 : Analysis of variance for response Y1.

Source	Sum of Squares	df	Mean Square	F-value	p-value
D	631.92	2	315.96	33.12	0.000
AxD	71.17	2	35.58	3.73	0.034
Residual	333.87	31	9.539		
Total	1036.95	39			

F value: variation between sample means / variation within the samples df:degree of freedom p-value: determine statistical significance in a hypothesis test

Table 5.15 : Observed (OV) and Predicted (FITS) values for response Y1.

Code	OV	FITS	R	Code	OV	FITS	R
MIP 1	23.50	26.34	-2.84	NIP 1	23.60	24.94	-1.34
MIP 2	29.80	29.31	0.49	NIP 2	32.20	26.41	5.79
MIP 3	20.90	22.74	-1.84	NIP 3	25.40	21.37	4.03
MIP 4	17.40	18.62	-1.22	NIP 4	18.60	17.96	0.64
MIP 5	24.20	25.55	-1.35	NIP 5	28.30	26.22	2.08
MIP 6	29.00	30.61	-1.61	NIP 6	32.50	27.71	4.79
MIP 7	20.50	18.86	1.64	NIP 7	15.50	18.20	-2.70
MIP 8	31.60	30.58	1.02	NIP 8	23.00	29.18	-6.18
MIP 9	24.00	25.96	-1.96	NIP 9	27.80	26.63	1.17
MIP 10	18.00	16.15	1.85	NIP 10	12.50	14.78	-2.28
MIP 11	30.80	30.15	0.65	NIP 11	23.50	27.25	-3.75
MIP 12	24.70	24.27	0.43	NIP 12	23.20	22.87	0.33
MIP 13	23.90	23.35	0.55	NIP 13	20.40	21.99	-1.59
MIP 14	21.70	22.14	-0.44	NIP 14	21.10	21.48	-0.38
MIP 15	28.50	28.44	0.06	NIP 15	28.10	29.12	-1.02
MIP 16	32.80	31.41	1.39	NIP 16	30.60	30.01	0.59
MIP 17	19.50	19.48	0.02	NIP 17	18.70	18.81	-0.11
MIP 18	19.10	19.67	-0.57	NIP 18	20.70	18.31	2.39
MIP 19	29.00	28.53	0.47	NIP 19	25.40	25.63	-0.23
MIP 20	32.10	28.85	3.25	NIP 20	27.30	29.53	-2.23

The data analysis indicated that the linear effect of D and the interaction AxD were significant at 5% level. It can be seen that crosslinker ratio (D) had the major effect with nine times to the next most significant functional monomer type×crosslinker ratio (A×D) interactively effect. Figure 5.20 was plotted by analyzing all MIP and NIP results. The analysis of the variables by ANOVA showed when the crosslinker ratio increased from 1 to 5 mol %, the time required for 90 percent of the released was shortened by an average of 8 hours. Additionally, as the crosslinker ratio increased,

the AA and IA responses varied from each other. At 1 and 3 mol % crosslinker ratios, IA based hydrogels prolonged releasing drug time. While crosslinker ratio was over 3 mol %, AA based hydrogels released 90 % drug in longer time.

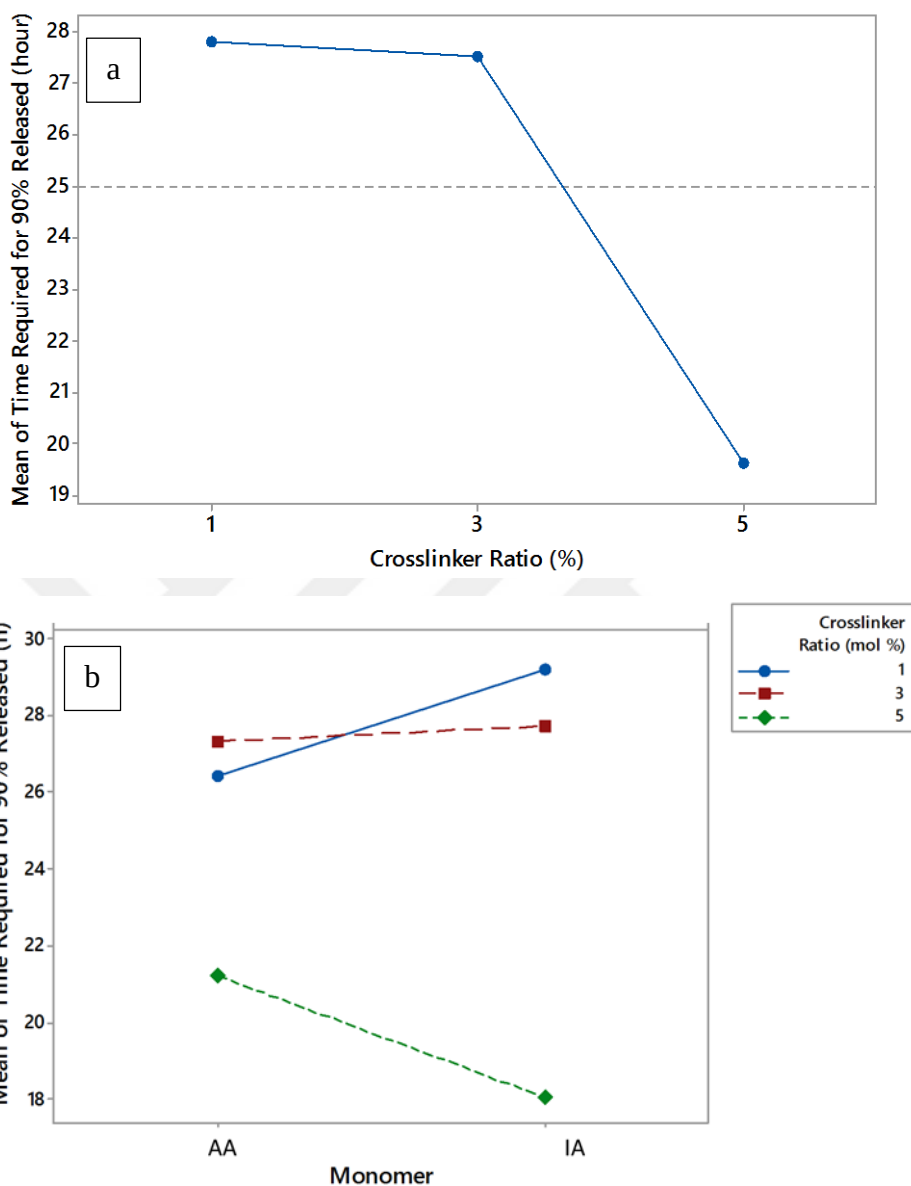


Figure 5.19 : Factorial plots for response Y1 **a.** main effects plot and **b.** interaction plots.

The positive coefficients obtained from the regression equation was related to the extending release time and the negative coefficients obtained from the regression equation was related to the shortening release time for 90 % of drug. At 10 h retardation in Y1 response was observed when crosslinker ratio was increased from 1 to 5 with IA as functional monomer (Table 5.16).

Table 5.16 : Regression coefficients for response Y1.

$Y1 = 24.994 + 2.831 D_1 + 2.544 D_3 - 5.375 D_5 - 1.383 A \times D AA_1 - 0.203 A \times D AA_3 + 1.586 A \times D AA_5 + 1.383 A \times D IA_1 + 0.203 A \times D IA_3 - 1.586 A \times D IA_5$
--

IA and AA showed opposite effect when they interacted with crosslinker ratio individually. The time required for 90% release increased with crosslinker ratio if the acrylic acid was used. On the other hand, when itaconic acid was used as the functional monomer, the increased crosslinker ratio reduced the time required for 90 % release.

5.3.1.2 Y2: Amount of drug released (%) in 4 h

The rapid release occurred at the first phase for approximately 4-6 h during the biphasic release behavior. One of our goals was to slow down the release rate by molecular imprinting method at the first phase release. The model calculated in polymer library was impressively suitable and exhibited 84.81 % prediction ability of the response variance (Table 5.17), and the factors assessed in the experimental design demonstrated consistent and quantitative interactions mainly and interactively with the Amount of Drug Released (%) from the samples. The FITS and Residual values were presented in Table 5.18.

Table 5.17 : Model summary for response Y2.

R-sq (%)	R-sq (adj) (%)	R-sq (pred) (%)
87.92	84.81	80.65

The main and interactively effects on the Y2 response in the model were presented in Table 5.19 for the polymer library. The main effects dominantly controlled the system for Y2 response. The most significant factor that had remarkable impact on the Y2 response was crosslinker ratio. Crosslinker ratio clarified 56.7% and crosslinker type clarified 21.1 % of the total sum of squares within the system (Table 5.19).

Table 5.18 : FITS and residuals for response Y2.

Code	OV	FITS	Residual	Code	OV	FITS	Residual
MIP 1	50.74	47.67	3.07	NIP 1	49.61	48.57	1.04
MIP 2	46.35	46.85	-0.50	NIP 2	47.33	49.05	-1.72
MIP 3	62.90	61.13	1.77	NIP 3	68.36	67.26	1.11
MIP 4	62.34	59.50	2.84	NIP 4	66.83	67.34	-0.51
MIP 5	59.04	60.54	-1.50	NIP 5	61.65	63.28	-1.64
MIP 6	46.39	45.67	0.73	NIP 6	50.67	47.86	2.80
MIP 7	62.00	62.33	-0.33	NIP 7	72.68	70.17	2.51
MIP 8	49.30	47.44	1.85	NIP 8	53.77	48.34	5.43
MIP 9	58.79	58.34	0.45	NIP 9	57.40	61.08	-3.67
MIP 10	68.33	64.12	4.21	NIP 10	71.36	70.25	1.11
MIP 11	45.97	44.84	1.13	NIP 11	47.19	47.04	0.15
MIP 12	48.98	50.22	-1.24	NIP 12	45.92	51.12	-5.20
MIP 13	53.75	56.21	-2.46	NIP 13	62.09	62.34	-0.26
MIP 14	46.48	50.03	-3.55	NIP 14	55.67	57.87	-2.20
MIP 15	45.04	46.70	-1.66	NIP 15	50.98	49.44	1.54
MIP 16	41.75	45.44	-3.69	NIP 16	43.68	46.34	-2.66
MIP 17	58.45	57.41	1.04	NIP 17	61.87	65.25	-3.38
MIP 18	51.14	54.65	-3.51	NIP 18	62.40	60.78	1.62
MIP 19	46.86	48.22	-1.36	NIP 19	50.57	50.42	0.15
MIP 20	47.20	44.49	2.71	NIP 20	51.01	47.24	3.77

Table 5.19 : Analysis of variance for response Y2.

Source	Sum of Squares	df	Mean Square	F-value	p-value
C	573.1	1	573.08	54.10	0.000
D	1542.3	2	771.15	72.80	0.000
E	160.8	3	53.61	5.06	0.006
C*D	137.4	2	68.72	6.49	0.004
Residual	328.4	31	10.59		
Total	2719.2	39			

The Amount of Drug Released (%) was linearly affected by the drug ratio (Figure 5.20 a). Adding drug to the monomer mixture in pre-polymerization step slowed the release rate during in vitro studies at the first 4-6 h. The polymers containing 5 mol % EGDMA released 66.16 % drug whereas polymers containing 5 mol % TEGDMA released drug 55.79 % in 4 hours. The crosslinker ratio effect on Y2 response was evaluated with crosslinker type due to interaction between crosslinker ratio and crosslinker type (Figure 5.20 b). Drug released in 4 h decreased from 56.34 to 51.97 % when imprint technique was applied (Figure 5.20a). This indicated that imprinting method had slightly effect on decreasing the cumulative release of the drug from the hydrogels.

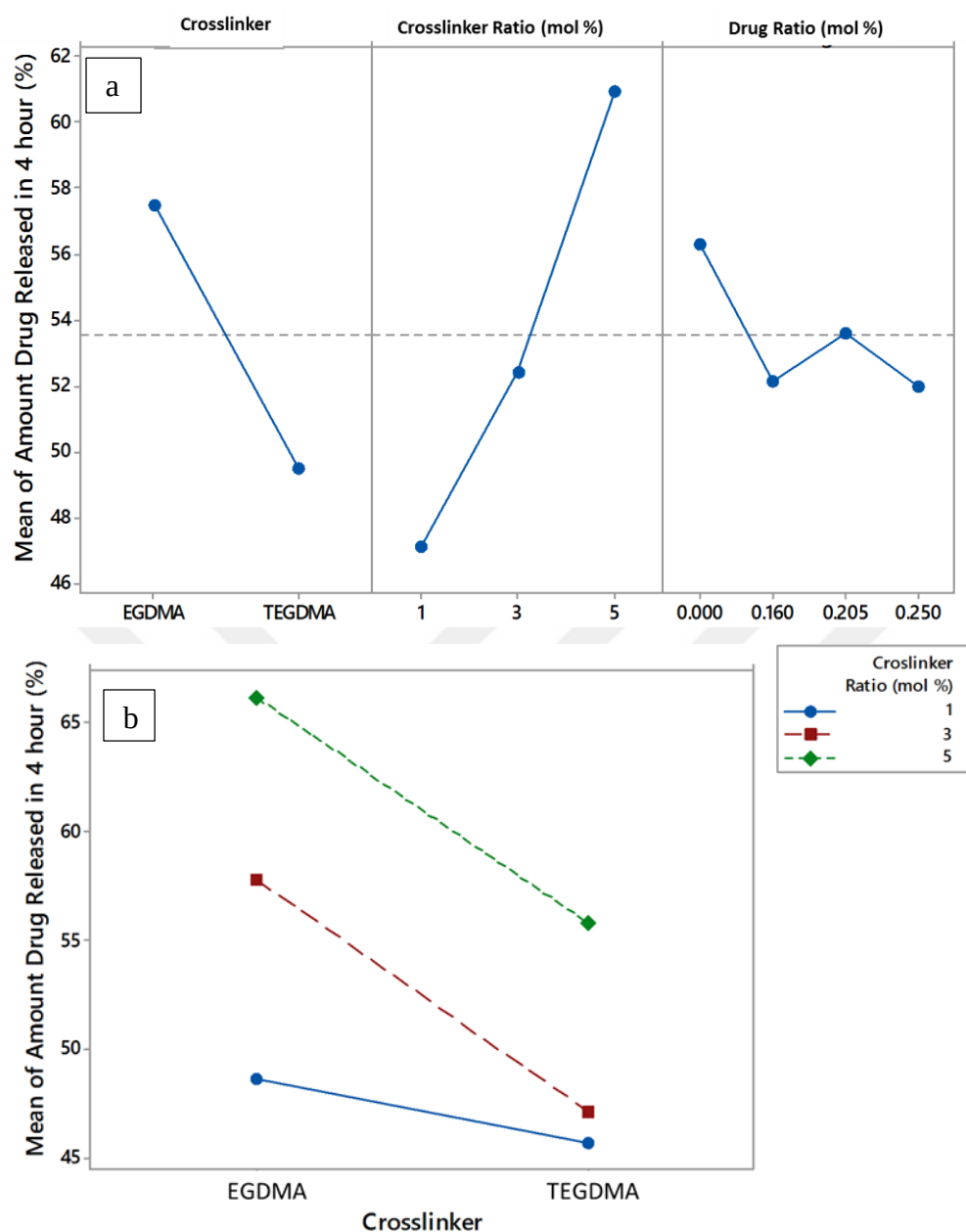


Figure 5.20 : Factorial plots for response Y2 **a.** main effects plot and **b.** interaction plots.

On the other hand, the effect of crosslinker type was linked with crosslinker ratio on Y2 response, interactively (Figure 5.20 b). Thus, the effect of crosslinker ratio was evaluated with crosslinker type. The effect of crosslinker type was more pronounced with increasing crosslinker ratio. For each crosslinker ratio, TEGDMA provided slow release rate compared to EGDMA. TEGDMA based hydrogels released less drug than EGDMA based hydrogels for each crosslinker ratio in 4 h as percentage. The factors which had negative coefficients obtained from regression equation helped to retard the release of the drug. As the crosslinker ratio increased, the difference in the % amount

of drug released in 4 hours between EGDMA and TEGDMA polymers became larger (Figure 5.20 b). Coefficients obtained from regression equation proved that the molecular imprinting method showed a significant effect on each ratio with reducing % amount released in 4 h (Table 5.20). The coefficients were -1.55 and +2.819 when drug ratio was 0.250% and without drug in pre-polymerization mixture respectively. Regression equation which showed the effects of the variables are given in the model, numerically.

Table 5.20 : Regression coefficients for response Y2.

$Y_2 = 53.518 + 3.990 \text{ EGDMA} - 3.990 \text{ TEGDMA} - 6.385 \text{ D}_1 - 1.08 \text{ D}_3 + 7.462 \text{ D}_5 + 2.819 \text{ E}_{0.000} - 1.34 \text{ E}_{0.160} + 0.08 \text{ E}_{0.205} - 1.55 \text{ E}_{0.250} - 2.538 \text{ CxD EGDMA}_1 + 1.342 \text{ CxD EGDMA}_3 + 1.196 \text{ CxD EGDMA}_5 + 2.538 \text{ CxD TEGDMA}_1 - 1.342 \text{ CxD TEGDMA}_3 - 1.196 \text{ CxD TEGDMA}_5$
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5.3.1.3 Y3: Total amount of released drug in 48 h

The total amount of released drug in 48 hours for the polymers was varied depending on the polymer composition. The studies demonstrated that the molecular imprinting was also effective on the increasing total amount of released drug [31,33,113,223]. It was found that imprinting method was dominantly effective in increasing the amount of drug released from the polymers. The R-Sq (adj) value was 86.14 %, indicated that, the five studied factors could describe 86.14 % of the variation in the total amount of released drug modelling (Table 5.21). The FITS and Residual values were calculated by ANOVA according to regression equation factors which had p value were eliminated and refitting was completed and presented in Table 5.22.

Table 5.21 : Model summary for response Y3.

R-sq (%)	R-sq (adj) (%)	R-sq (pred) (%)
90.40	86.14	80.06

The most significant factors in the model were presented in Table 5.23 for the polymer libraries including both MIPs and NIPs. The results indicated that the linear effects of A, B, C and the interactions of A×B and D×E were significant in the 5% level. B×E and B×D interactions were not estimated and removed from regression by ANOVA. It was suggested that D×E had the major influence on the Y3 response. After D×E effect, the second significant effect was A on total amount of released drug in 48 hours.

It can be seen that two factor interaction including drug ratio effect dominated the system, D×E, accounted for 58.96 % of the total sum of squares in the data.

Table 5.22 : Observed (OV) and Predicted (FITS) values for response Y3.

Code	OV	FITS	R	Code	OV	FITS	R
MIP 1	3.75	3.78	-0.03	NIP 1	2.47	3.06	-0.58
MIP 2	4.74	4.67	0.07	NIP 2	4.12	3.93	0.18
MIP 3	0.90	-0.09	0.99	NIP 3	1.04	0.09	0.94
MIP 4	0.95	1.05	-0.10	NIP 4	1.11	0.97	0.14
MIP 5	1.92	2.09	-0.17	NIP 5	1.14	1.16	-0.02
MIP 6	4.49	4.52	-0.04	NIP 6	3.79	3.78	0.00
MIP 7	1.21	0.90	0.31	NIP 7	0.87	0.82	0.04
MIP 8	7.32	6.78	0.54	NIP 8	7.31	6.06	1.25
MIP 9	2.89	3.05	-0.17	NIP 9	1.86	2.12	-0.27
MIP 10	1.59	2.92	-1.33	NIP 10	1.32	3.10	-1.78
MIP 11	3.56	4.44	-0.88	NIP 11	2.74	3.70	-0.96
MIP 12	5.28	5.30	-0.02	NIP 12	4.39	4.58	-0.19
MIP 13	1.40	1.43	-0.04	NIP 13	1.57	1.62	-0.04
MIP 14	1.20	0.82	0.38	NIP 14	0.88	0.74	0.14
MIP 15	2.45	2.73	-0.28	NIP 15	2.27	1.80	0.47
MIP 16	4.67	5.15	-0.48	NIP 16	3.67	4.43	-0.75
MIP 17	3.23	3.82	-0.59	NIP 17	3.75	3.74	0.01
MIP 18	1.66	1.28	0.38	NIP 18	2.01	1.47	0.54
MIP 19	8.29	7.44	0.85	NIP 19	7.75	6.70	1.05
MIP 20	4.31	3.70	0.62	NIP 20	2.58	2.76	-0.18

Table 5.23 : Analysis of variance for response Y3.

Source	Sum of Squares	df	Mean Square	F-value	p-value
A	14.57	1	14.72	25.72	0.000
B	25.30	2	12.33	22.33	0.000
C	4.14	1	4.14	7.32	0.012
A×B	4.25	2	2.12	3.75	0.037
D×E	93.96	6	15.66	27.65	0.000
Residual	15.29	27	0.57		
Total	159.35	39			

Although it is mentioned in the literature that the functional monomer and the template interaction in the pre-polymerization step will influence the shape and number of binding sites in the polymer [71,217], the effects of crosslinker ratio and drug ratio were dominantly observed in this study. IA caused more drug release than AA (Figure 5.21 a). This was due to the more –COOH groups in IA which acted with drug functional groups [213,224]. On the other hand, it was asserted that TEGDMA lead to the increase amount of drug released in 48 h from the hydrogels. This was not

surprising due to the crosslinker which affect the shape of binding sites and force of the hydrogen bonds in the polymer [49,172,225].

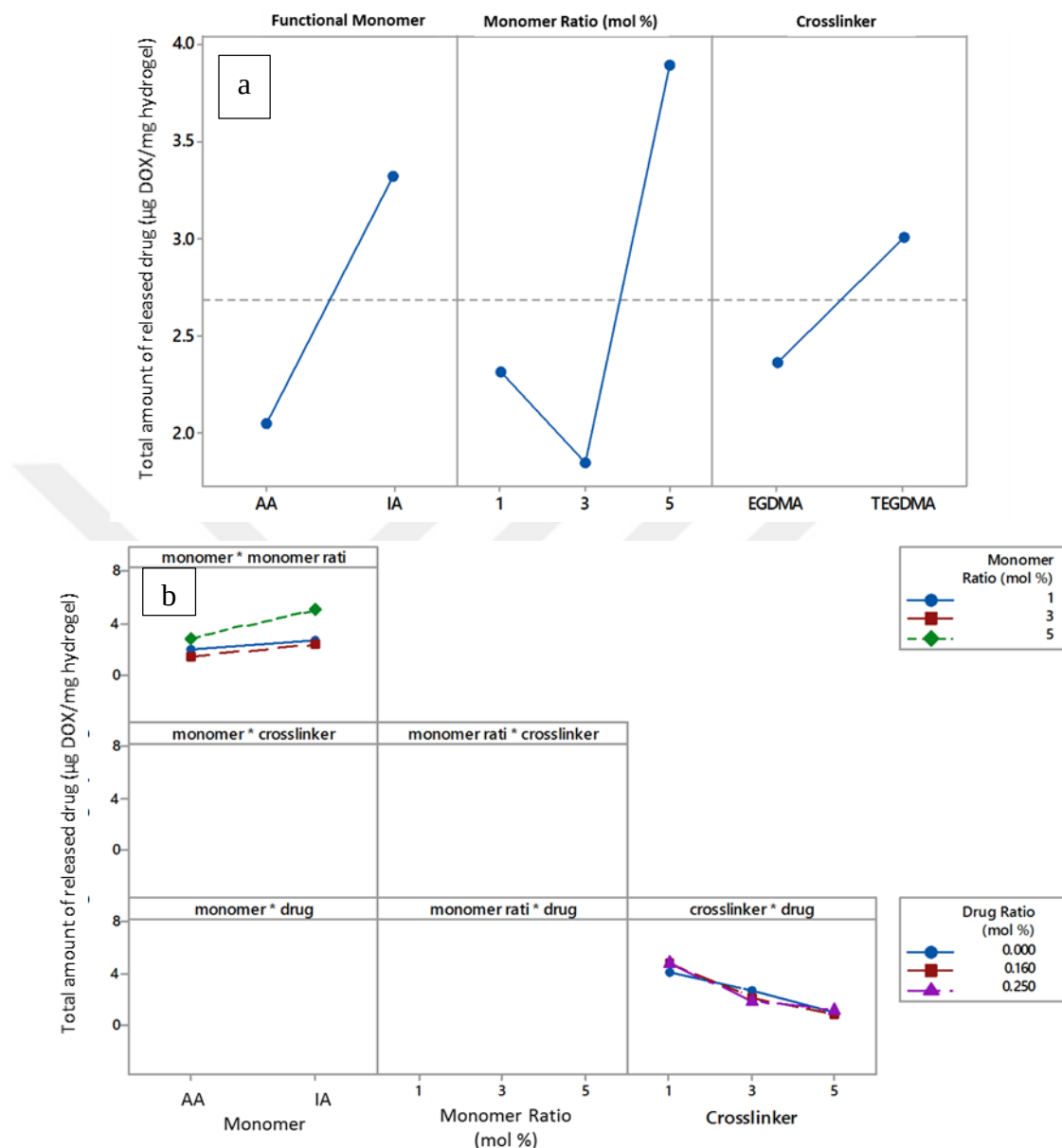


Figure 5.21 : Factorial plots for response Y3 **a.** main effects plot and **b.** interaction plots.

The crosslinker ratio itself affected the rigidity of the formed cavities and flexibility of the polymers [65,226,227]. The amount of DOX influenced Y3 response depending on the crosslinker ratio used to prepare the polymers (Figure 5.21b). The template showed a significant influence when crosslinker ratio was 1, whereas in the case of 5% crosslinker was used the influence of molecular imprinting effect decreased drastically on the response Y3 leading to a decrease in drug release. Therefore, the existence of template and its interaction with the moiety of the crosslinker in the pre-polymerization

mixture had an influence on the amount of released drug in 48 hours of the MIP. If the two factors that showed the highest effect in the MIPs (crosslinker ratio and functional monomer ratio) were considered, the amount of released drug increased when the functional monomer ratio increased and the crosslinker ratio decreased.[89] Moreover, when TEGDMA was used as crosslinker, amount of the released drug increased with increasing monomer ratio. This was due to the fact that TEGDMA was a long chain crosslinker and the cavities shaped with TEGDMA have been more flexible and also the chain length between two crosslinking points have been longer. In the literature, the best results were observed when the crosslinker and functional monomer ratios are similar or their ratios close to each other [168,169,228,229]. The monomer ratio showed an important influence when monomer type is selected as IA, whereas in the case of 5 mol % monomer ratio was used, the released drug in 48 hour increased two times compared with 1 mol % monomer ratio. When IA replaced with AA, the releasing amount increased slightly with increasing monomer ratio (Figure 5.21 b).

As shown in Table 5.24, positive coefficients indicated an increase in the amount of released drug, while negative coefficients revealed lower amount of released drug. The positive constant obtained from regression equation for IA (+0.636) as functional monomer factor demonstrated that polymers which were synthesized with IA released more drug than polymers containing AA monomer in per unit weight of polymer. IA-5 (+0.426) and TEGDMA (+0.322) exhibited positive effect on the increasing amount of released drug in 48 h. The coefficient was -5.745 for crosslinker ratio 1 with drug ratio of 0.205 (Table 5.24).

Table 5.24 : Regression coefficients for response Y3.

$Y3 = 2.686 - 0.636 AA + 0.636 IA - 0.371 B_1 - 0.834 B_3 + 1.205 B_5$ $- 0.322 EGDMA + 0.322 TEGDMA + 0.272 A \times B_{AA\ 1} + 0.154 A \times B_{AA\ 3}$ $- 0.426 A \times B_{AA\ 5} - 0.272 A \times B_{IA\ 1} - 0.154 A \times B_{IA\ 3} + 0.426 A \times B_{IA\ 5}$ $+ 1.427 C \times E_{1_0.000} + 2.167 C \times E_{1_0.160} - 5.745 C \times E_{1_0.205} + 2.151 C \times E_{1_0.250}$ $+ 0.108 C \times E_{3_0.000} - 0.451 C \times E_{3_0.160} + 1.040 C \times E_{3_0.205}$ $- 0.698 C \times E_{3_0.250} - 1.535 C \times E_{5_0.000} - 1.717 C \times E_{5_0.160} + 4.704 C \times E_{5_0.205}$ $- 1.453 C \times E_{5_0.250}$

5.3.1.4 Y4: n values obtained from Korsmayer-Peppas equation

The release kinetics was used to define the release mechanism. The regression coefficients (R^2) were calculated from linearly fitted plots and the release constants were calculated from the slope of the fitted plots. The preliminary studies showed that

Korsmeyer-Peppas plot best fit with release kinetics (R^2 0.9481–0.9997) (Table 5.35) rather than Higuchi plot for our molecular imprinting system (Table 5.38).

Korsmeyer and Peppas plots were employed to study the release data. Thus, it was indicated that the control mechanism of the hydrogels was different for all the formulations. The release pattern suited the biphasic release mechanism, characterized by relatively fast release followed by the slow release. The first releasing step was investigated at this section. The obtained release exponents n_1 and n_2 for polymers including NIPs and MIPs were presented in Table 5.25 and 5.26 as minimum and maximum values.

Table 5.25 : Korsmeyer-Peppas n_1 values for NIPs and MIPs with different monomer-crosslinker types for the first step.

	n_1 values MIP	n_1 values for NIP
AA-EGDMA	0.8238-1.220	0.9094-1.425
AA-TEGDMA	0.6039-0.895	0.7026-1.370
IA-EGDMA	0.6850-0.914	0.6873-1.154
IA-TEGDMA	0.4991-0.631	0.7152-0.931

Table 5.26 : Korsmeyer-Peppas n_2 values for NIPs and MIPs with different monomer-crosslinker types for the second step.

	n_2 values MIP	n_2 values for NIP
AA-EGDMA	0.3473-0.618	0.3579-0.435
AA-TEGDMA	0.2727-0.475	0.3249-0.703
IA-EGDMA	0.3289-0.398	0.3740-0.670
IA-TEGDMA	0.4146-0.497	0.4703-0.608

Release of DOX from both NIPs and MIPs in 0.9 % NaCl solution was attributed essentially to super case II transport which indicates relaxation and diffusional control for $n > 0.89$ and non-Fickian transport indicates the relaxation of the polymers for $0.45 > n > 0.89$ at the first step [230]. The n_1 values decreased when the imprinting process was applied to the hydrogels. The n_2 values were found to be low due to the slow drug release in the second phase. The range of n_2 values was narrowed by applying molecular imprinting method (Table 5.35). It was indicated that imprinting technique was significantly effective on changing releasing kinetic mechanism. n_2 values were commonly beyond the 0.45 value of Korsmeyer Peppas approach. It can be realized from Table 5.25 that there was a significant difference in the n_1 values of monomer and crosslinker pairs between NIPs and MIPs. The polymers including IA-

TEGDMA as monomer and crosslinker pair had lower n values than the others for both NIPs and MIPs. IA-TEGDMA based hydrogels released drug via non-fickian diffusion. AA-EGDMA had higher n values than the others for both NIPs and MIPs. AA-EGDMA based hydrogels released drug via super case II transport mechanism. A review about release kinetic indicated that n value below of 0.5 value was diffusional controlled release [180]. Additionally, molecular imprinting technique decreased the differences between the first and second drug release phases by controlling release patterns.

Table 5.27 : Model summary for response Y4.

R-sq (%)	R-sq (adj) (%)	R-sq (pred) (%)
79.12	74.55	67.49

It was observed that imprinting method was very effective in release mechanism of the hydrogels at the first step. The model considered from hydrogel library was good enough and exhibited 74.55 % estimate ability of the response variance Y4 (Table 5.27).

Table 5.28 : Analysis of variance for response Y4.

Source	Sum of Squares	df	Mean Square	F-value	p-value
A	0.5161	1	0.5161	46.36	0.000
C	0.3035	1	0.3035	27.27	0.000
E	0.3554	3	0.11847	10.46	0.000
B*C	0.1560	2	0.07801	7.01	0.003
Residual	0.3562	32	0.01113		
Total	1.7060	39			

There were main and interactively factors which influenced the drug release mechanism (Table 5.28). It was confirmed that A, C,E factors had linear effects and the interaction B×C had significantly effect on the n_1 values of the hydrogels in the 5% level. B×E, C×D, and D×E were not estimated and removed from the model which is expressed for Y4 response. The factors which had p value higher than 0.05 was removed from regression equation and then the FITS and Residual values were calculated by ANOVA and presented in Table 5.29.

Table 5.29 : Observed (OV) and Predicted (FITS) values for response Y4.

Code	OV	FITS	R	Code	OV	FITS	R
MIP 1	0.824	1.047	-0.223	NIP 1	1.081	1.219	-0.139
MIP 2	0.975	0.960	0.015	NIP 2	1.188	1.080	0.107
MIP 3	1.220	1.099	0.121	NIP 3	1.386	1.219	0.167
MIP 4	0.977	0.908	0.069	NIP 4	0.909	1.080	-0.171
MIP 5	0.833	0.894	-0.061	NIP 5	1.425	1.165	0.260
MIP 6	0.876	0.872	0.004	NIP 6	0.935	0.992	-0.058
MIP 7	0.895	0.820	0.076	NIP 7	1.105	0.992	0.113
MIP 8	0.749	0.681	0.069	NIP 8	0.759	0.853	-0.094
MIP 9	0.604	0.667	-0.063	NIP 9	0.911	0.938	-0.028
MIP 10	0.719	0.733	-0.013	NIP 10	0.703	0.853	-0.151
MIP 11	0.798	0.786	0.012	NIP 11	0.900	0.907	-0.007
MIP 12	0.889	0.874	0.015	NIP 12	0.982	1.046	-0.064
MIP 13	0.914	0.925	-0.012	NIP 13	1.018	1.046	-0.029
MIP 14	0.763	0.735	0.028	NIP 14	0.894	0.907	-0.013
MIP 15	0.685	0.689	-0.004	NIP 15	0.890	0.961	-0.071
MIP 16	0.519	0.507	0.012	NIP 16	0.715	0.680	0.035
MIP 17	0.602	0.646	-0.045	NIP 17	0.738	0.819	-0.081
MIP 18	0.499	0.559	-0.060	NIP 18	0.735	0.680	0.055
MIP 19	0.631	0.698	-0.067	NIP 19	0.931	0.819	0.112
MIP 20	0.591	0.462	0.128	NIP 20	0.790	0.734	0.056

The IA based hydrogels had lower n_1 value than the AA hydrogels. The coefficient is -0.1136 for IA and -0.0918 for TEGDMA and -0.1304 for 0.205 % drug ratio (Table 5.30). The results showed that existing drug as template in pre-polymerization solution triggered a change in drug release kinetic by altering n value.

Table 5.30 : Regression coefficients for response Y4.

$Y4 = 0.8085 + 0.1136 \text{ AA} - 0.1136 \text{ IA} + 0.0918 \text{ EGDMA} - 0.0918 \text{ TEGDMA}$ $+ 0.1412 \text{ E}_{0.000} + 0.0205 \text{ E}_{0.160} - 0.1304 \text{ E}_{0.205} - 0.0313 \text{ E}_{0.250}$ $+ 0.0643 \text{ BxC 1_EGDMA} - 0.0643 \text{ BxC 1_TEGDMA} + 0.0103 \text{ BxC 3_EGDMA}$ $- 0.0103 \text{ BxC 3_TEGDMA} - 0.0746 \text{ BxC 5_EGDMA} + 0.0746 \text{ BxC 5_TEGDMA}$

The diffusional exponent n_1 obtained from Korsmeyer-Peppas Equation underwent an impressive decrease when the molecular imprinting method was applied to prepare polymers. n values which were greater than 0.89 represented super case II transport mostly for non-imprinted hydrogels most likely driven by chain relaxation and swelling of hydrogel (Figure 5.22 a). Similar results obtained for IA and TEGDMA based hydrogels: non-fickian diffusion occurred when n value was between 0.45 and 0.89. Diffusion and relaxation rates can be comparable. In this case, the drug release behavior can be regarded as a superposition in both cases. For IA-TEGDMA monomer crosslinker pair showed values of n fairly higher than 0.5, which proposes that the drug

was released predominantly by non-fickian mechanism (including diffusion and relaxation) in the first step. TEGDMA showed similar DOX releasing kinetics for all functional monomer ratios and represented non-Fickian diffusion statistically. However, releasing mechanism of EGDMA based hydrogels transformed from super case II to non-fickian transport with increasing monomer ratio. This was attributed that EGDMA interacted with DOX non-specifically and increasing monomer ratio to prevent EGDMA-DOX interaction (Figure 5.22 b). It was indicated that releasing mechanisms were different for EGDMA and TEGDMA beased hydrogels.

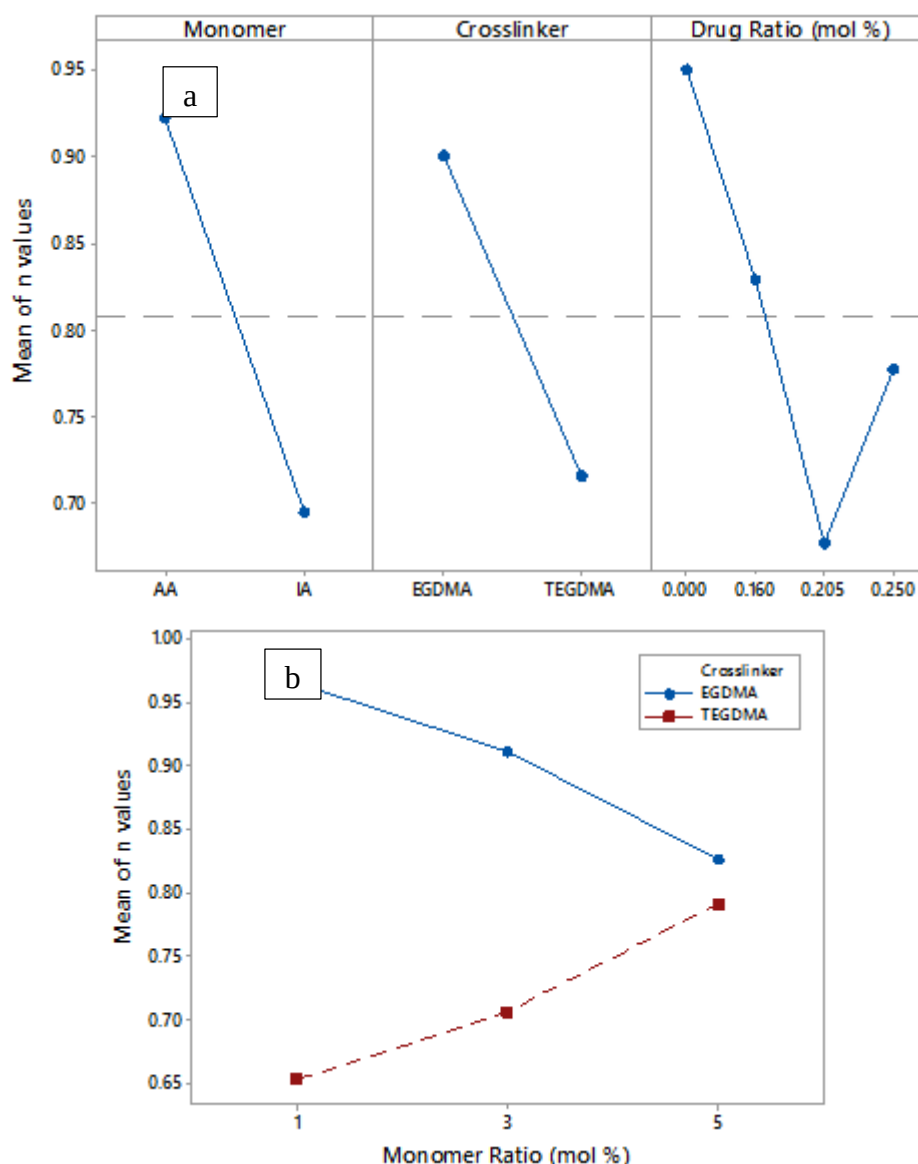


Figure 5.22 : Factorial plots for response Y4 **a.** main effects plot and **b.** interaction plots.

5.3.1.5 Final optimization with multiple response optimizer and validation of model

“Multiple response optimizer” was used to optimize formulations by considering one or more responses, jointly. It offered an optimal combination from input variables within the MINITAB 17 and acted as a composite desirability component and the combinations were ranged according to potential responses [169]. The goal of each response can be selected as maximum, minimum or as a target value. It was assumed that all responses have equal efficient and the optimization of the formulation is identified by maximizing default value (d).

The formulations used for the development of the validation groups were as represented in the Figure 5.24. The response optimizer in MINITAB 17 suggested a probable combination of the factors which jointly optimized four responses (Y1) as 29.21 hour, (Y2) as 44.34 %, (Y3) as 7.44 µg DOX/mg polymer, (Y4) as 0.6982 and provided variables values of functional monomer type as IA, functional monomer ratio as 5 mol %, crosslinker type TEGDMA, crosslinker ratio 1 mol % and drug ratio as 0.160 mol % that can yield the desired requirements ($d=0.8510$) of the imprinting process (Figure 5.23 a). Additionally, if Y2 was minimum and Y4 was also minimum the other responses were not optimized; The functional monomer type was IA, the functional monomer ratio was 3 mol %, the crosslinker type TEGDMA, the crosslinker ratio was 5 mol % and the ratio of the drug was 0.250 mol % (Figure 5.234 b). When Y3 was maximized and Y4 was optimized as minimum; the functional monomer ratio is 5 mol %, the crosslinker type is TEGDMA, the crosslinker ratio is 3 mol %, and the ratio of the drug is 0.205 mol % (Figure 5.23 c).

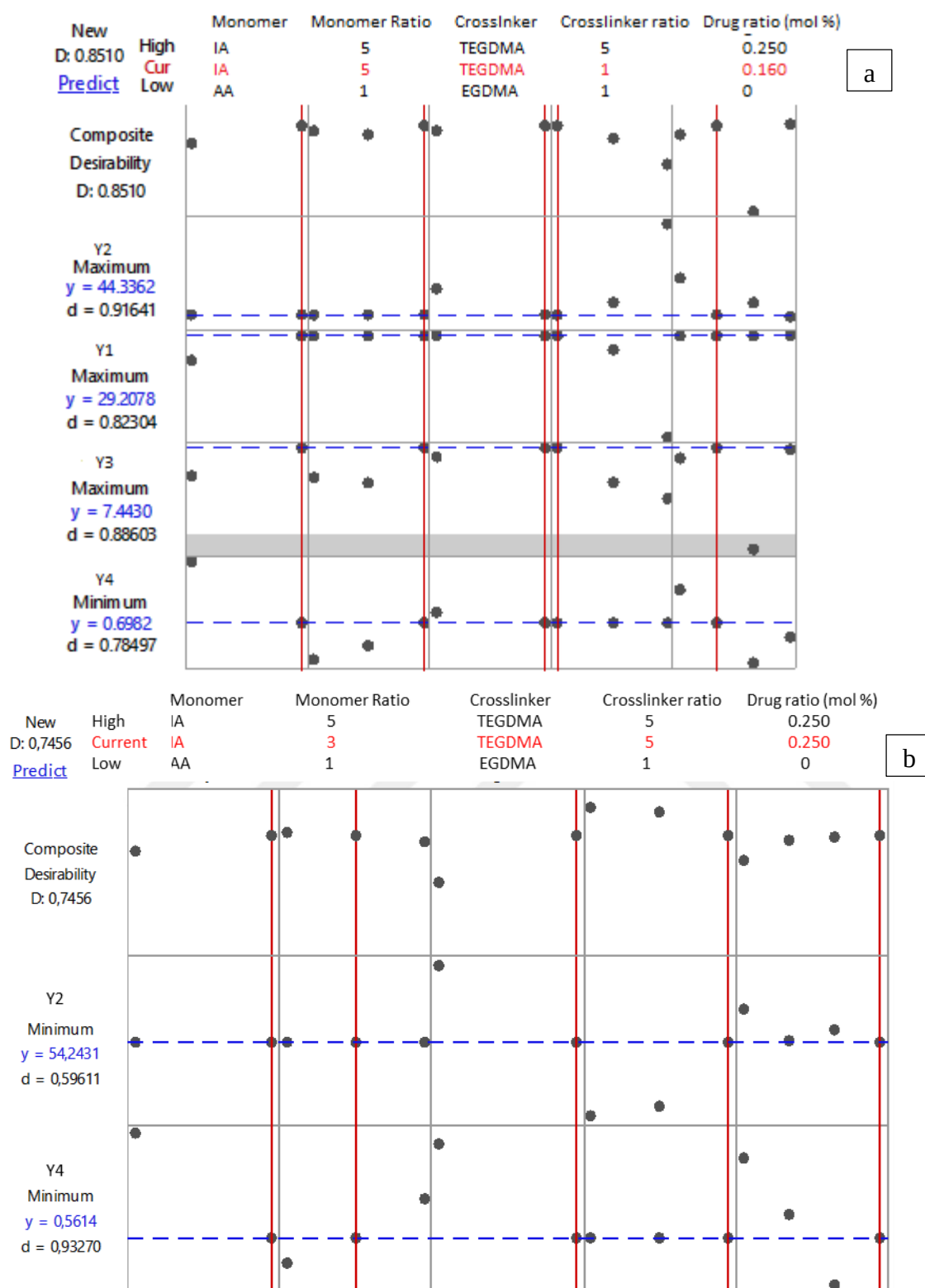


Figure 5.23 : Response optimizer solutions for the input variable combinations.

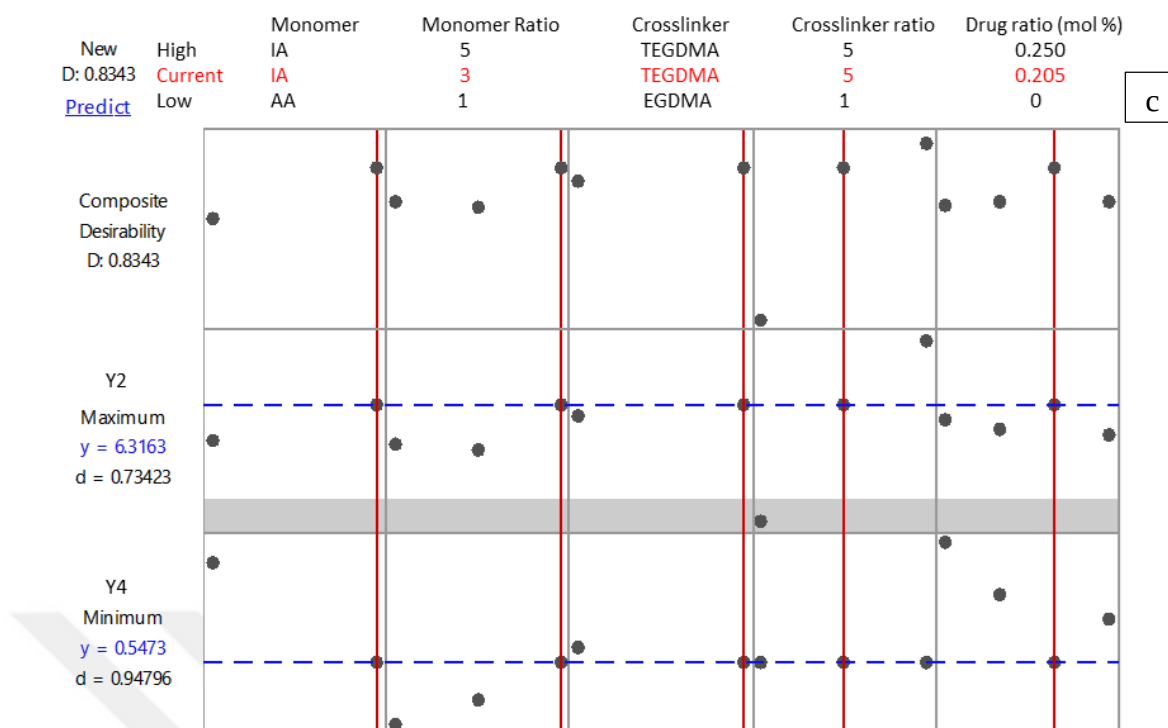


Figure 5.23(Continued): Response optimizer solutions for the input variable combinations.

The results of the developed formulations were shown in Table 5.31. In Table 5.31, it appeared that the errors associated with responses determined by statistical and experimental methods can be seen.

Table 5.31 : Experimental results for hydrogels obtained from response optimizer and experiments.

Response	Formulation 1		Formulation 2		Formulation 3	
	Predict	Experimental	Predict	Experimental	Predict	Experimental
Y1	29.21	28.53	Not	-	Not	-
Error(%)		(2.38)	optimized		optimized	
Y2	44.34	46.86	54.24	47.32	Not	-
Error(%)		(5.38)		(12.76)	optimized	
Y3	7.44	8.29	Not	-	6.3163	6.240
Error(%)		(10.25)	optimized			(1.22)
Y4	0.6798	0.6313	0.5614	0.5538	0.5473	0.5166
Error(%)		(7.68)		(1.38)		(5.94)

The difference between the predicted and experimental values was within the limits of 5 mol %, as required by the model for formulation 3. However, the other errors which bigger than 5 mol % maybe approve acceptable for formulation 1 and 2. Consequently, it can be stated that statistical analysis and experimental modelling are useful for determining the optimal polymerization compositions for DOX imprinted hydrogel. And three formulations were valid in determining the optimal reaction conditions for

the imprinted hydrogel synthesis using DOX as template. The formulation 3 was chosen for in-vivo experiments for the next phase of the project.

5.3.2 Characterization and drug loading and release studies

The hydrogels prepared according to the given formulation in Table 5.12, were characterized by several methods. The characterization results were shared and discussed in this section. Hydrogels were coded as numerical such as 1,2,3 etc. And so, the imprinted and non-imprinted states were indicated as MIP 1-NIP 1, MIP 2-NIP 2, etc.

5.3.2.1 FTIR studies

The structure of the hydrogels was confirmed by FTIR spectral analysis. After the completion of the polymerization, the characteristic peak of -C=C at 1637 cm^{-1} did not completely dissapeared, but the presence of 3405 cm^{-1} and 1720 cm^{-1} confirmed the groups -OH and C=O in the copolymer structure, respectively. After the washing of the hydrogels, the characteristic peak of -C=C at 1637 cm^{-1} dissapeared. The results show that the imprinted and non-imprinted hydrogels have been successfully synthesized. All FTIR spectra were given in Appendix A (Figures A.4-A.8).

5.3.2.2 NMR studies

The ^{13}C NMR spectrums of the both imprinted and non-imprinted hydrogels were given in Appendix B (Figures B1 and B2). ^{13}C NMR has been used to estimate the structure of the hyrogels.

Results indicated that there was not a chemical shift between 128-138 ppm related with DOX molecules for imprinted hydrogel. Also, a chemical shift did not observed between 100-150 ppm due to C=C doule bond. ^{13}C NMR spectra exhibited the following signals at (δ , ppm): 15 [-CH_3]; 17 [-CH_2]; 44 [$\text{-CH}_2\text{-C=O}$]; 54-57 [C-CN from solvent]; 66 [$\text{O-CH}_2\text{-}$]; 177 [-C=O] for both imprinted an non-imprinted hydrogels.

5.3.2.3 DSC studies

Thermal properties of DOX-imprinted and non-imprinted hydrogels were determined by DSC (Table 5.32). All dry hydrogels, showed a single glass transition temperature. The T_g was observed $104.6\text{-}122.5\text{ }^\circ\text{C}$ for the imprinted hydrogels and $97.9\text{ - }124.7\text{ }^\circ\text{C}$

for non-imprinted hydrogels. T_g of the HEMA-based hydrogels were similar to the values found in the literature which is between 97-117 °C [138,231].

The fact that, absence of the characteristic melting point of doxycycline hyclate at 184 °C in the DSC thermograms of molecular imprinted hydrogels (Figure 5.24) indicated the removing of the drug successfully after synthesise via washing procedure. The lowest T_g was observed by DSC for hydrogels made of TEGDMA, while the highest T_g corresponded to EGDMA based hydrogels (Table 5.32). This was described by the fact that the presence of relatively longer crosslinker gained the hydrogels with a greater flexibility and caused to decrease of T_g value of the hydrogels. T_g values were higher than that of the non-imprinted hydrogels as expected for complexes achieved through hydrogen bond formation between polymer chains, leading to higher T_g values for imprinted hydrogels. Considering the fact that hydrogels containing IA as functional monomer had higher T_g values than the AA based hydrogels clearly showed formation of apparent hydrogen bonding between the chains obtained with IA and also T_g increased with increasing monomer ratio in the polymer composition.

Table 5.32 : T_g values of NIPs and MIPs.

F. monomer	F. Monomer ratio (mol %)	Crosslinker	Crosslinker Ratio (mol %)	Drug ratio (mol %)	MIP T_g	NIP T_g
AA	1	EGDMA	1	0.250	112.3	112.9
AA	5	EGDMA	1	0.160	112.9	110.9
AA	1	EGDMA	5	0.160	118.9	111.1
AA	5	EGDMA	5	0.250	122.2	113.6
AA	3	EGDMA	3	0.205	116.8	97.9
IA	1	EGDMA	1	0.160	109.9	106.6
IA	1	EGDMA	5	0.250	122.5	103.8
IA	5	EGDMA	1	0.250	116.4	108.6
IA	3	EGDMA	3	0.205	118.7	122.1
IA	5	EGDMA	5	0.160	122.4	124.7
AA	1	TEGDMA	1	0.160	104.6	100.8
AA	5	TEGDMA	1	0.250	108.3	112.7
AA	5	TEGDMA	5	0.160	113.0	100.0
AA	1	TEGDMA	5	0.250	106.7	98.6
AA	3	TEGDMA	3	0.205	112.2	109.7
IA	1	TEGDMA	1	0.250	111.4	110.0
IA	5	TEGDMA	5	0.250	119.4	115.7
IA	1	TEGDMA	5	0.160	108.9	106.8
IA	5	TEGDMA	1	0.160	111.0	109.4
IA	3	TEGDMA	3	0.205	109.4	110.5

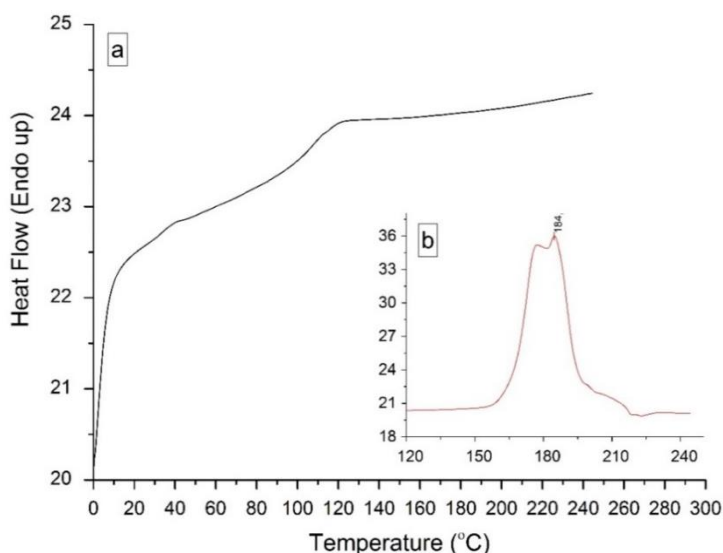


Figure 5.24 : DSC profiles of (A) hydrogels synthesized with DOX after washing. (B) doxycycline hyclate powder.

Similar results were obtained by Garay and coworker. They studied with poly(N-isopropylacrylamide) with poly(acrylic acid) and poly(methacrylic acid). They indicated that reduction of the flexibility of the polymer chains caused to increase of T_g to higher values [232].

5.3.2.4 Morphology of hydrogels

Scanning electron microscopy was used to investigate the morphology of the hydrogels. SEM images of the hydrogel 5 and 20 (both MIP and NIP) were given in Figures 5.25 and 5.26 with low and high magnification. The hydrogels also appeared to be smooth in size. It is indicated that there was a uniform surface and cross-sectional area for both NIP and MIP. It can be seen that the pores were successfully emptied by washing solution and they indicated on the Figure 5.25 a by arrow mark. Figures 5.25 c is cross-sectional micrograph of imprinted hydrogel and showed that pores were also present on the cross-sectional surface of the imprinted hydrogel. Pores with an average diameter of approximately 11-18 μm were confirmed by SEM on the surface and cross-sectional area for TEGDMA based imprinted hydrogel (Figure 5.25 c). The size of the cavity measured from SEM image was consistent with the theoretical size of DOX (11 μm). There was no porous structure on the surface and cross-section area of non-imprinted TEGDMA based hydrogel (Figure 5.25 b and d).

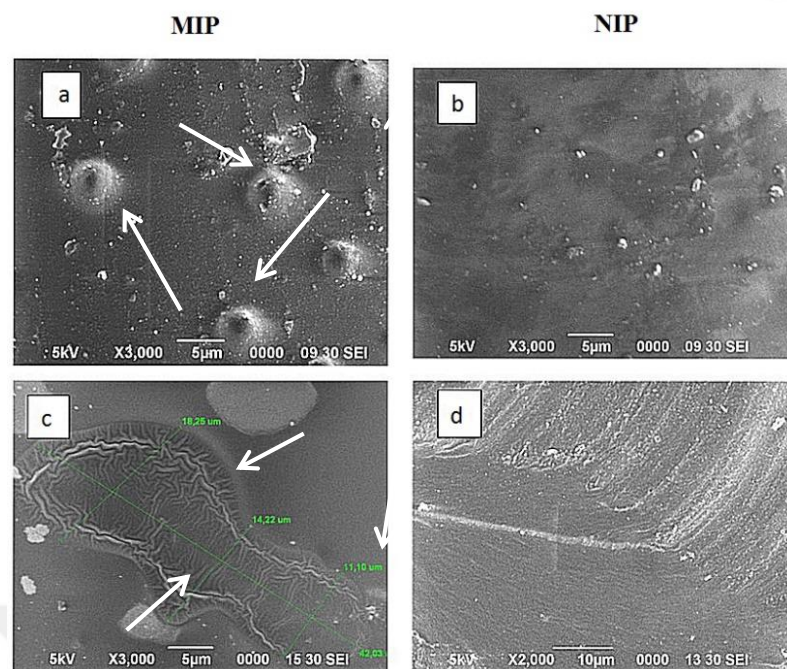


Figure 5.25 : SEM images of IA-TEGDMA based NIP and MIP as surface (a and b) cross sectional area (c and d).

Figures 5.26 shows SEM images of DOX imprinted and non-imprinted hydrogels synthesized with EGDMA. A cavited layer can be clearly distinguished for imprinted hydrogels with very low pore sizes (arrow marks, Figure 5.26a) and a smooth surface for non-imprinted sample (Figure 5.26b). As seen from Figure 5.26c and 5.26d, both the EGDMA based MIP and NIP had a smooth cross-section surface. When the diameters of the cavities were compared, the cavities in the hydrogels containing EGDMA were smaller than the hydrogel containing TEGDMA. These holes were much smaller than the size of a single DOX molecule.

The difference between EGDMA and TEGDMA based samples may associate with template-monomer complex packaged in the MIP matrix. After the removal of DOX from the hydrogels, the structure of pore channels was created in the MIP network, which is clearly favorable for the template molecules transport and mass embedding. Yu et al. prepared molecularly imprinted polymers and investigated their surface properties with SEM analysis. They realized that MIP had also porous surface and also they proved that the MIPs had denser structure than NIP via porosity measurements. This case allowed to succesful adsorption performance [233].

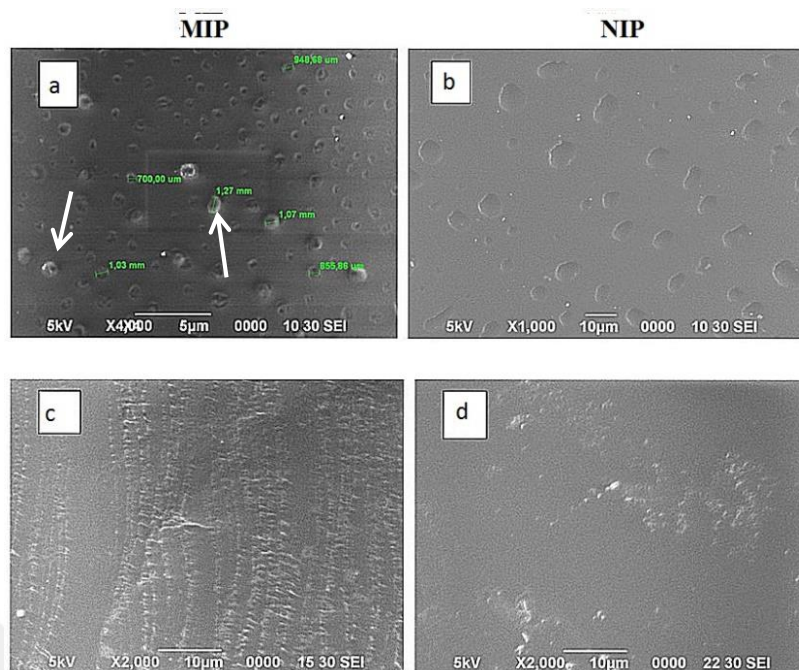


Figure 5.26 : SEM images of AA-EGDMA based NIP and MIP as surface (a and b) cross sectional area (c and d).

5.3.2.5 Cell toxicity of the hydrogels

The cytotoxicity assays are the most crucial biocompatibility test for many polymeric materials. Cell toxicity was determined by the inhibition of cell proliferation. The purpose of this study was to evaluate whether there is a cytotoxic response of NIP and MIP (5 mol % TEGDMA, 3 mol % IA and 0.250 DOX presenced during polymerization) samples on retinal pigment epithelium cells (RPE cells).

According to results, the cell viability increased starting from the first day of the cultivation till the end of 7th day in all samples. After 7 days of incubation, cell viability decreased in all samples including only cell (OC). It might be related with the excessive cell proliferation and as a result of cell detachment. Therefore, it can be said that this decline was not due to the toxic effect of polymers since we can observe the same decrease in both cell containing groups. After 14 and 21 days of incubation, the cell viability increased in all samples again. To conclude, as it can be seen from the results that these hydrogels are not cytotoxic to RPE cell line and there is no significant difference in cell proliferation between MIP and NIP polymers on RPE cells (Figure 5.27).

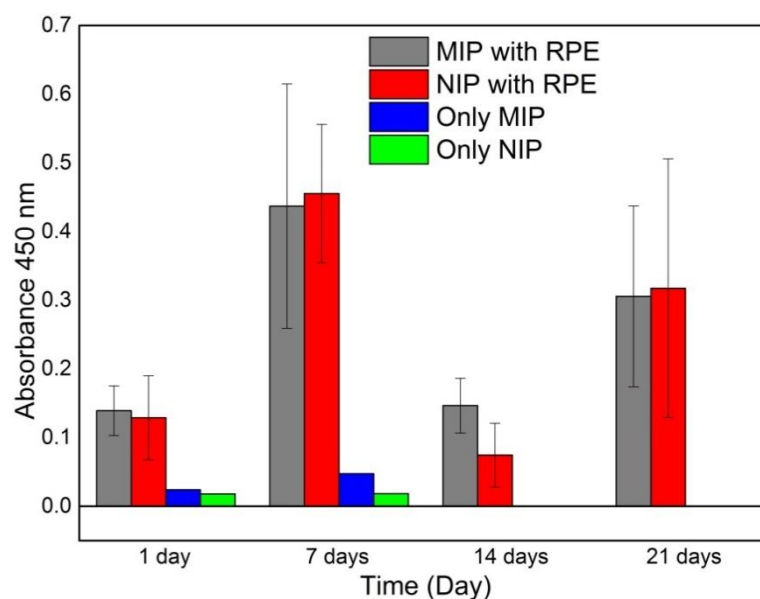


Figure 5.27 : Cell viability test response of RPE cells in the imprinted and non-imprinted hydrogels.

5.3.2.6 Swelling behavior of the hydrogels

Swelling behavior is an important parameter on understanding drug release mechanism. It can be described by the Flory Rehner theory. This theory indicates that in equilibrium, swelling conditions of a crosslinked polymer depend on two forces: thermodynamic force of mixing and elastic force of the polymer chains [31]. From thermodynamic point of view, the total Gibbs free energy is equal to zero and Gibbs free energy at equilibrium is expressed as Equation 5.4.

$$\Delta G_{total} = \Delta G_{elastic} + \Delta G_{mixing} \quad (5.4)$$

It should be stated that another free energy term must be incorporated to reflect the complexation contribution of multiple polymer chains because of imprinting when the swelling of the hydrogel affected by the complexation of chains via various non-covalent interactions with the multi-functional template molecule. It is expected that strong and multiple non-covalent interactions in polymer chains with numerous functional groups have been resulted in lower expansion of the imprinted polymers than non-imprinted polymers. Notably, this effect can be clearly seen from the swelling results in Figure 5.28. For imprinted hydrogels that represent the template depended swelling changes, the free energy that participates in the template-chain complexation need to be included as a term and evaluated as Equation 5.5 [234]:

$$\Delta G_{total} = \Delta G_{elastic} + \Delta G_{mixing} + \Delta G_{imprinting\ complexation} \quad (5.5)$$

It is worthy to know that ionic strength had a significant role in the hydrogel swelling behavior. The ionic strength effect on the reversible swelling behavior of MIPs and NIPs was investigated by using the swelling agents such as, 1 mM loading DOX solution (pH=3.9), 0.9 % NaCl (pH=7.0) solution and distilled water (pH=7.0). 1mM DOX solution was also prepared to emulate the loading conditions for dry hydrogel swelling. Distilled water and 0.9% NaCl played a key role in the releasing medium with the same pH but with different ionic strength. In order to evidence the ionic strength effect on the swelling ratio of the hydrogels, the temperature was kept constant at 37 °C [235].

The swelling behavior of the hydrogels in distilled water, 0.9 % NaCl solution and 1 mM DOX solution was compared and presented in Figure 5.28 for NIPs and MIPs, respectively. In experimental studies, it was observed that hydrogels transferred from drug solution to distilled water and 0.9 % NaCl solution absorbed more water with increasing pH which can be seen in Figure 5.28. The IA-TEGDMA based non-imprinted hydrogels absorbed more water than other hydrogels due to their more hydrophilic structure [49]. As expected, the swelling ratio decreased as the crosslinker ratio increased. Under the loading conditions, functional groups of the hydrogels were in the protonated state for AA ($P_{ka} 4.25 > pH 3.90$ of loading solution) and partially deprotonated state IA ($P_{ka1} 3.85 < pH 3.89$ of loading solution). Once the hydrogels were transferred to the release medium (0.9 % NaCl solution or distilled water), fully deprotonation induced and this led to the initiation of swelling. It can be stated that polymer chains containing ionic groups led the increasing of swelling ratio due to the stronger dissolution of the ions in the medium. It was expected that the swelling at alkaline solution increased with ionization of $-COOH$ groups on the polymer chain. High crosslinker ratio caused to limit the diffusion of water molecules into the hydrogel network. This was attributed to the increase in the electrostatic repulsion (anion-anion repulsion) between $-COO^-$ groups which caused the hydrogel absorbs more water and also high amount of crosslinker led to decrease of ionization of carboxyl groups. Therefore, it was estimated that experimental conditions such as pH and ionic strength may utilize essential results on the drug release profiles.

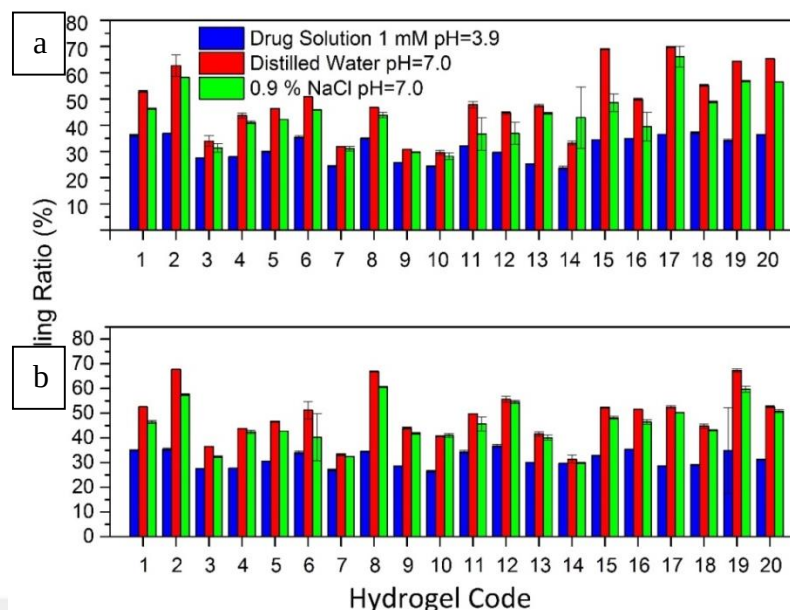


Figure 5.28 : Influence of the medium on swelling ratios of a. non-imprinted (NIPs) and b. imprinted hydrogels (MIPs).

It is obvious that swelling ratio slightly depended on the salt which added to the distilled water. The swelling content of the hydrogels noticeably were lower in 0.9% NaCl compared to the measured in distilled water (Figure 5.28 a and b). This was attributed to the dominantly ionic strength differences between swelling mediums which caused a swelling change. This is a well-known phenomenon which affects generally ionic hydrogels. Osmotic swelling pressure differences between the hydrogel and swelling medium reduced dramatically because of the charge-screening effect of the added cations which weakened the electrostatic repulsion. After the ionization of the hydrogels, the electrostatic repulsion gave rise to extension of anionic hydrogel matrix. When NaCl was added into the solution, the anionic groups of the hydrogel was screened via the counterions (Na^+ ions) in the swelling medium. Ionic strength increased efficiently and electrostatic repulsion decreased between the two anions ($-\text{COO}^-$) thus the swelling ratio of the hydrogel decreased. Sakthivel et al. showed that higher electrostatic repulsion of negatively charged ions lead to higher swelling ratios [236].

5.3.2.7 Oxygen permeability

Traditional contact lens materials are obtained from poly(2-hydroxyethyl methacrylate) (pHEMA) which is biocompatible and have sufficient optical, swelling and mechanical properties. It is also necessary to provide the oxygen permeability to

protect the health of the cornea [27]. The hydrogel material must be permeable to oxygen in order to preserve cornea health [171].

Oxygen permeability profile of contact lens was important due to oxygen requirement of cornea. Oxygen permeability is defined as Dk , where D is the diffusivity of the material and k is the solubility of the material. The relationship between the equilibrium water content (EWC) and the Dk of conventional hydrogels was inspected and were represented in Figure 5.29. It is importantly noted that this relationship can be applied only to the hydrogel in which the oxygen transport takes place only through the water, not through the material. So, it is not suitable for use for gas permeability calculations in silicon-based hydrogels [237].

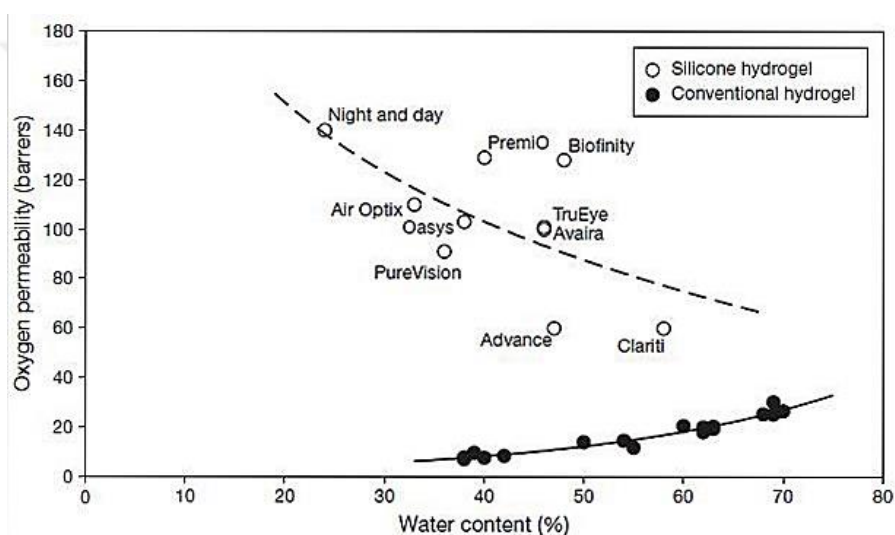


Figure 5.29 : Relationship between oxygen permeability and equilibrium water content for conventional hydrogel lenses (solid curve) and silicone hydrogels (dashed curve) [237].

The oxygen permeability results with swelling ratios were represented in Figure 5.30. The oxygen permeability of both imprinted and non-imprinted hydrogels calculated from equilibrium water content was upper than minimum required value. In the literature the minimum required oxygen permeability is indicated as $Dk/L_0=5.0$ barrer/mm where is the L_0 is dry hydrogel thickness [27]. It is indicated that all materials exhibited adequate oxygen permeability performances. Imprinted hydrogels oxygen permeabilities were slightly lower than non-imprinted counterparts in general due to lower water absorption capacities.

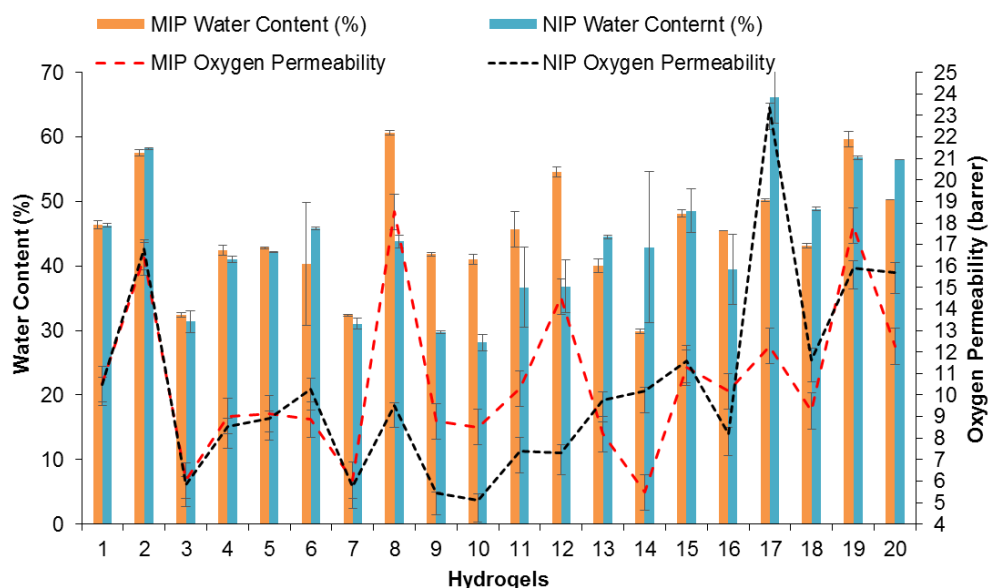


Figure 5.30 : Oxygen permeability and water content relation for both NIPs and MIPs.

5.3.2.8 Structural analysis of the hydrogels

It should be stated that in the template transport studies, structure of the hydrogels should be evaluated. But, it should also be noted that structural change is not the only criteria for drug transport from MIPs and NIPs. The porosity of the hydrogels can be classified as macroporous, microporous and nanoporous [238]. If an imprinted hydrogel is synthesized in the absence of a solvent, a hydrogel with small pores similar to the template size will be formed without micro or macro porous. After immersing hydrogels in a solvent, the drug transport is effected by the mesh size between the polymer chains and imprinting effect. The interactions between crosslinked hydrogel and drug are influenced by the nature of the components, and also by changes in its nanostructure [239].

Volume fraction in swollen state data were plotted and were represented in Figure 5.31 a and b. The $V_{2,s}$ difference between non-imprinted and imprinted hydrogels were pronounced for hydrogels 17, 18 and 20 in the loading solution which had IA and TEGDMA as functional monomer and crosslinker in their structures. The imprinted hydrogels synthesized with IA and TEGDMA generally had higher volume fraction in swollen state than the IA-TEGDMA including non-imprinted hydrogels in the loading solution. This difference may be related with effect of imprinting on the polymerization. The addition of DOX in the pre-polymerization mixture can effect the designing of polymer chains by interacting with both crosslinker and monomer. It may

led to make polymer chains less mobile and decreased the water absorption capacity in the loading solution. And this led to be higher $V_{2,s}$ value than non-imprinted counterparts. Ali and coworker reported that imprinting altered structural properties of the hydrogels and these cases changed drug release behavior of the hydrogels [26]. Vaughan observed that hydrogels prepared via living radical polymerization had higher $V_{2,s}$ value and better releasing performance than imprinted hydrogel which was prepared via conventional radical polymerization. He indicated that better imprinting led to extent smaller during swelling [139].

The Figure 5.31a clearly disclosed the narrow range of polymer volume fraction of the hydrogels. This is a suggestion that the loading results did not only vary due to structural parameters of the network but also affinity between drug molecules and functional groups was important. In the loading solution, low crosslinked hydrogels had slightly lower $V_{2,s}$ than high crosslinked networks, as expected. Increasing the crosslinker ratio caused an increase in $V_{2,s}$ value which can be seen in Figure 5.31 a (hydrogel 3,4,7,10,13,14,17,18 were higher crosslinking hydrogels). Furthermore, Hydrogel 17 and 18 had less $V_{2,s}$ value than the other high crosslinked networks in which IA and TEGDMA was used as functional monomer and crosslinker. As known, low crosslinked hydrogels allowed to absorb more water than highly crosslinked hydrogels. $V_{2,s}$ value was related with water absorption capacities of the hydrogels and when hydrogel swelled to a smaller extent, it caused to higher $V_{2,s}$. More crosslinked points decreased swelling of the hydrogels and increased $V_{2,s}$ value.

If Figure 5.31b are discussed, it was seen that the addition of higher amount of functional monomer led to a decrease in $V_{2,s}$ (hydrogel 2,4,8,10,12,13,17,19 had higher amount of functional monomer) in the releasing medium. It was indicated that the presence of more amount of functional monomers decreased the $V_{2,s}$ due to ionization of functional groups on the polymer chains. Thus, it confirmed that functional monomer concentration effected the diffusion path with increasing volume of the hydrogel. It should be mentioned that lower crosslinker ratio allowed to absorb more water and as result of this $V_{2,s}$ value was smaller for hydrogel 2,8,12,19 than the other high crosslinked hydrogels. The other common point of these hydrogels was that they had 5 mol % by mol functional monomer on their structures. From our results, it was also realized that $V_{2,s}$ decreased with changing of swelling medium from 1 mM DOX solution to 0.9 % NaCl solution (Figure 5.31 a and b).

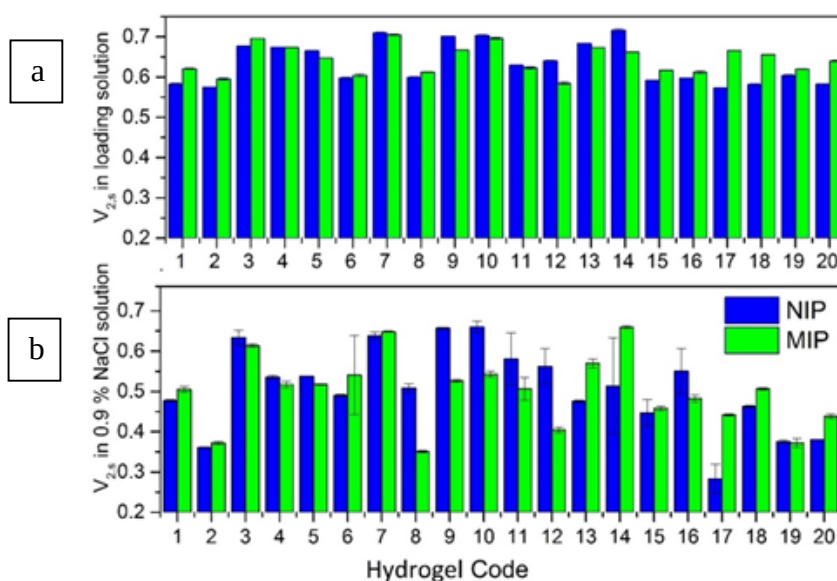


Figure 5.31 : Volume fraction in swollen state for imprinted and non-imprinted hydrogels in (a) loading and (b) releasing mediums.

The increase of chain mobility led to an increase in swelling and decrease in $V_{2,s}$. Hydrogels had ionizable functional groups on their structures with a varied ratios and it was suggested that hydrogels which had higher functional monomer ratio can absorb more water because of the ionization of carbonyl groups in the releasing medium. The flexibility of the hydrogels basically governed by the functional monomer and crosslinker. Swelling caused relaxation and enlargement of the polymer chains as well as decreasing the polymer volume fraction.

ξ , and $\bar{M}_c$ values for both NIPs and MIPs were summarized in Figure 5.32(a and b) in the loading solution. It was found that mesh size of the hydrogels were not strongly varied with imprinting. The molecular weight between two crosslinks was small for the hydrogels (both NIPs and MIPs) 3,4,5,7,9,10,13,14 which had higher amount of crosslinker on their structure (3 and 5 mol %). Their mesh sizes were also smaller than the other hydrogels. The crosslinking decreased mesh size and increased $V_{2,s}$, as expected. The mesh size was inversely proportional to $V_{2,s}$. The mesh size difference between the imprinted and non-imprinted hydrogels were maximum for hydrogel 1,2,15,16,17,18,19,20; imprinted hydrogels had lower mesh size than non-imprinted counterparts. Except hydrogel 1 and 2, the others had TEGMA as crosslinker and hydrogels from 16 to 20 contained IA as functional monomer. The nature of the crosslinker appeared to effect hydrogel network structure by changing mesh size and molecular weight between two crosslinking. The TEGDMA seemed to influence the formation of polymer chains and associated crosslink points in the hydrogel resulting

in a slightly lower mesh size than non-imprinted counterparts. This agrees with the results, in which imprinted hydrogels produced with the TEGDMA had a lower polymer volume fraction in swollen state in the loading medium (this result was seen more appereantly with IA) than hydrogels synthesized with EGDMA. In this case, it may suggest that TEGDMA-based hydrogels were more tightly, held drug molecules during drug loading process because of the small mesh size (Figure 5.33a).

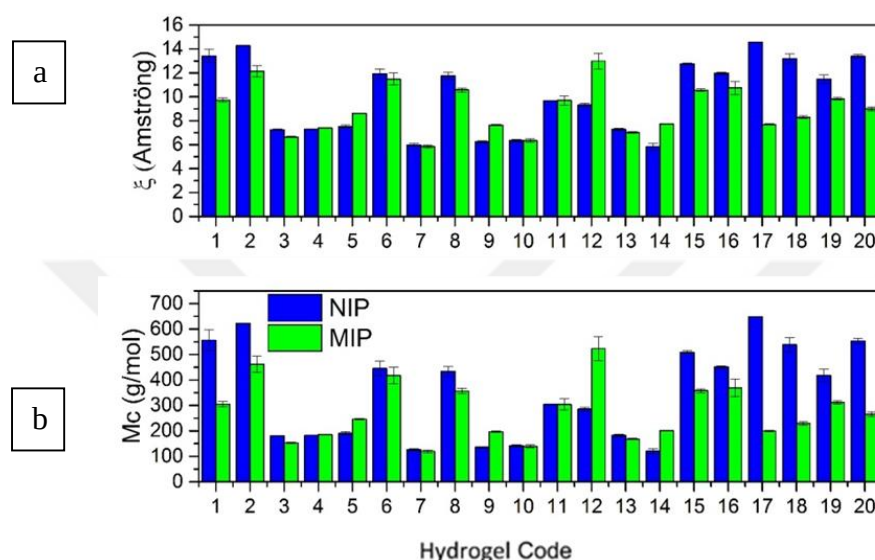


Figure 5.32 : Flory-Huggins network parameters of NIPs and MIPs in loading solution.

Our results showed that steric hindrance and high affinity may cause to a tighter mesh structure when imprinting process applied. The mesh size of the hydrogel matrix considerably effected the drug release properties via developing affinity binding sites in the cavities. This result was attributed to the effect of imprinting on structural organization of number of functional monomers in the template during polymerization which could mean a more open network available for transport of drug molecules. In spite of this, it could be supposed that the drug retarded compared to all the other systems. The imprinting hindered drug transportation for this kind of hydrogels. Additionally, crosslinked structure and mobility of the hydrogel chains after removing drug as template molecule by washing might cause steric hinderance or “screening effect” and limit the drug transport in release and loading stages [172].

Our results were supported by other published results. Ali and coworker studied with Hyaluronic Acid as template in order to prepare imprinted hydrogels. And they found that smaller mesh size retarded the particule diffusion in the imprinted hydrogel [26]. Singh et al. showed that lower mesh size blocked the penetration of water molecules

into the hydrogel matrix and decreased the amount of released drug [240]. Venkatesh and coworkers studied about the effect of imprinting on structures of the hydrogels. They offered that low $V_{2,s}$ values in the releasing medium mean that more open network available due to drug release [172].

5.3.2.9 DOX release behavior of the hydrogels in different solutions

In this study, the releasing data were collected for all NIPs and MIPs after 48 h. These data which presented in Figure 5.33, showed the total amount of released DOX in 0.9 % NaCl solution and distilled water. We know that DOX molecule was in the form of either a zwitter ion or a cation in loading solution in pH=3.9 and a zwitterion in the distilled water and 0.9 % NaCl solution in pH 7.0. As mentioned earlier, swelling behavior of the hydrogel and solubility of the drug was pH sensitive. Hydrogen bonding between DOX and functional monomers decreased in releasing medium. Since the swelling ratio and mesh size increased, the bond strength reduced. This resulted in divergence between the high affinity binding sites and DOX molecules and changing ionic forms of both hydrogel and drug molecule. On the other hand, it was seen that the amount of released drug increased in distilled water which had same pH and different ionic strength with 0.9 % NaCl solution. The common ion effect caused a decrease in solubility of the drug in the release medium as well as a decrease in drug released amount in 0.9 % NaCl solution. Additionally, the results displayed that the total amount of DOX released from small mesh sized hydrogels remains low (Hydrogel 3, 4, 5, 7, 9, 10, 13, 14).

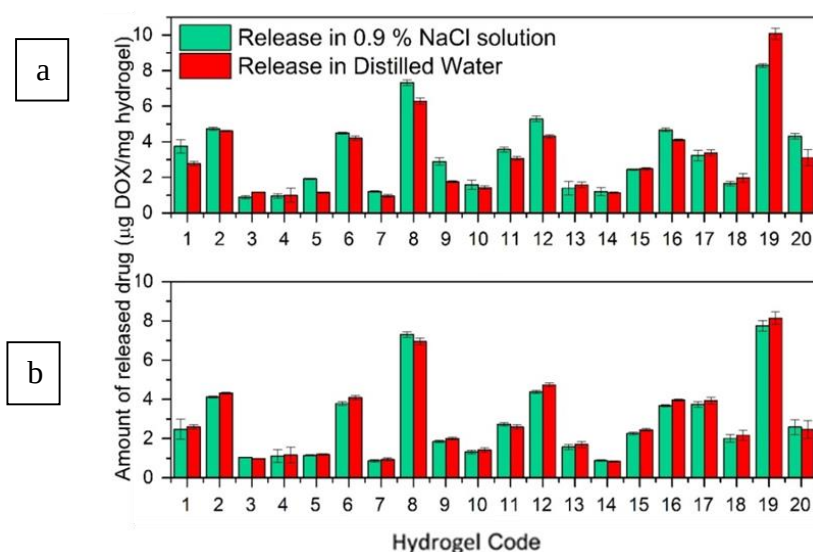


Figure 5.33 : Amount of DOX released from both NIPs and MIPs.

Solubility and dissolution rate of the doxycycline hyclate in different mediums changed depending on the common ion effect. The studies showed that dissolution rate of doxycycline hyclate were not effected by common ion effect (disolution rate joseph bogardus). However, solubility of the drug slightly decreased in 0.9 % NaCl solution. Joseph Bogardus and coworkers indicated that the common ion effect of chloride restricted the dissolution of the thermodynamically stable form of the drug molecules in the solvent [241].

Another important knowledge was that, the size of DOX is about 11 Å and this was comparable to the mesh size of both NIPs and MIPs. For this reason, it was unlikely that the DOX particles in the hydrogel were collected in the bulk phase during loading [242].

5.3.3 Drug loading studies

Released and loaded doxycycline hyclate as $\mu\text{g DOX/mg dry hydrogel}$ were obtained from the calibration curve given in Figure C1.

Several preparation factors affect the binding abilities of MIPs. In our study, we investigated the effects of five different factors on the performance of MIPs. The loading capacities of NIPs and MIPs were measured by UV-Vis spectroscopy at 274 nm and summarized in Table 5.33. The loading capacity results were also given in Figure 5.34.

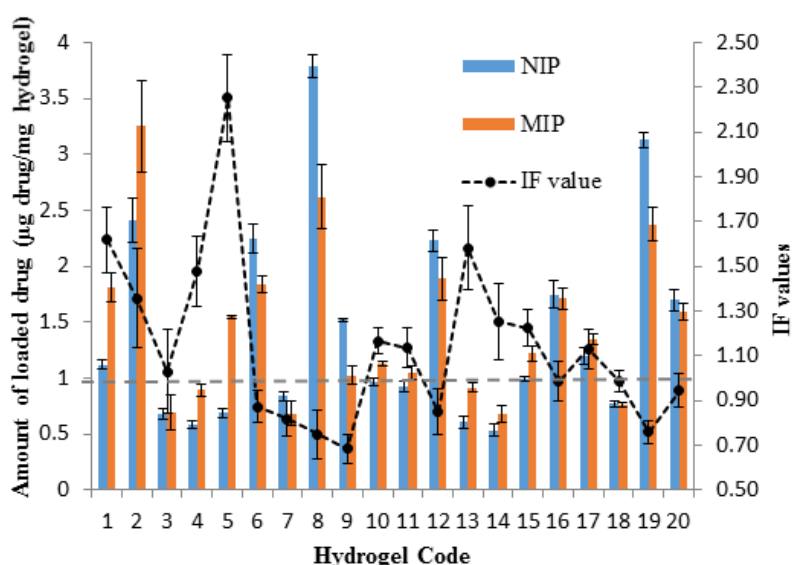


Figure 5.34 : Amount of loaded DOX by the non-imprinted and imprinted hydrogels by immersion in 0.1 mM DOX solution. The dashed grey line shows the reference point for IF value.

The results suggested that the different hydrogel contents had significant effect on the loading properties of both NIPs and MIPs. The results indicated that hydrogels with different monomer-crosslinker composition showed different loading properties. Many of the imprinted hydrogel loaded more drug than non-imprinted counterparts as an advantage.

In this part of the study, the results for AA based hydrogels have been presented. The AA-EGDMA based MIPs exhibited relatively higher degree of doxycycline hyclate binding capacity with respect to non-imprinted counterparts (Figure 5.34). The loadings were statistically different for $p < 0.05$. Namely, imprinted hydrogel showed on average $3.25 \mu\text{g}/\text{mg}$ binding and the non-imprinted ones on average $2.41 \mu\text{g}/\text{mg}$ binding for hydrogel 2 (IF = 1.36). Decreasing 5-fold the proportion of AA led to reduction of the loading capacity from 2.41 to $1.12 \mu\text{g}/\text{mg}$ for non-imprinted (IF = 1.62). Significant differences were observed for imprinted counterpart (from 3.25 to $1.81 \mu\text{g}/\text{mg}$ hydrogel) for hydrogel 1. Afterwards, the monomer:template ratio (M:T ratio) changed from 31.25 to 4. As it can be seen in Figure 5.35, decreasing the functional monomer ratio resulted in a decrease in the loading capacity of the hydrogels. However, imprinting efficiency increased with increasing IF. On the other hand, increasing 5-fold the proportion of EGDMA lowered the loading capacity from 1.12 to $0.68 \mu\text{g}/\text{mg}$ hydrogel for non-imprinted hydrogel and also decreased the loading capacity of the imprinted hydrogel from 1.81 to $0.69 \mu\text{g}/\text{mg}$ hydrogel (hydrogel 3 IF=1.03). Both IF value and loading capacities were dropped off. Furthermore, increasing AA proportion 5-fold (hydrogel 4) decreased non-imprinted hydrogel loading capacity ($0.58 \mu\text{g}/\text{mg}$ hydrogel) and increased loading capacity of the imprinted counterpart ($0.89 \mu\text{g}/\text{mg}$ hydrogel). The IF value was equal to IF=1.48. The M:T ratio for this hydrogel was equal to 20. Increasing both the crosslinker and monomer ratio enhanced the loading capacity of the imprinted hydrogel. When the crosslinker and monomer ratio was applied as 3 mol %, M:T was equal to 14.63 and IF value reached to a maximum value of 2.25 for hydrogel 5. The results plotted in Figure 5.35 and 5.36 revealed that both AA-EGDMA based imprinted and non-imprinted hydrogels had high DOX loading capacity at low crosslinker and high functional monomer ratios. But the amounts of rise and fall were not in equal proportions and might be a result of imprinting.

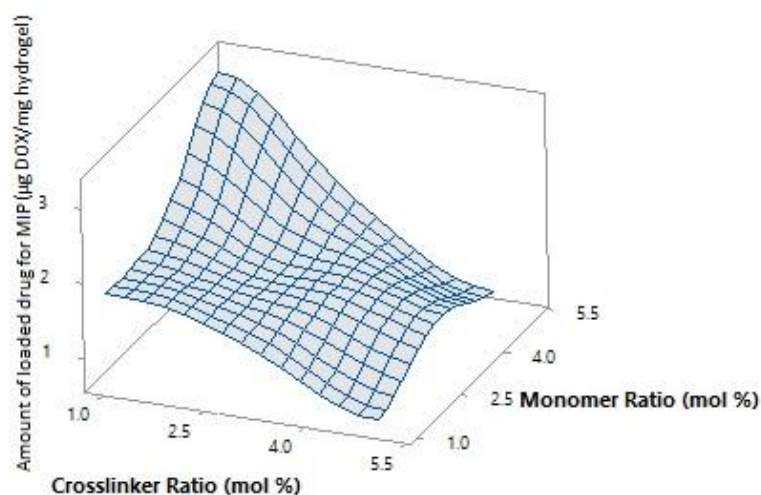


Figure 5.35 : 3D surface plot for crosslinker ratio-monomer ratio effect on AA-EGDMA based imprinted hydrogels.

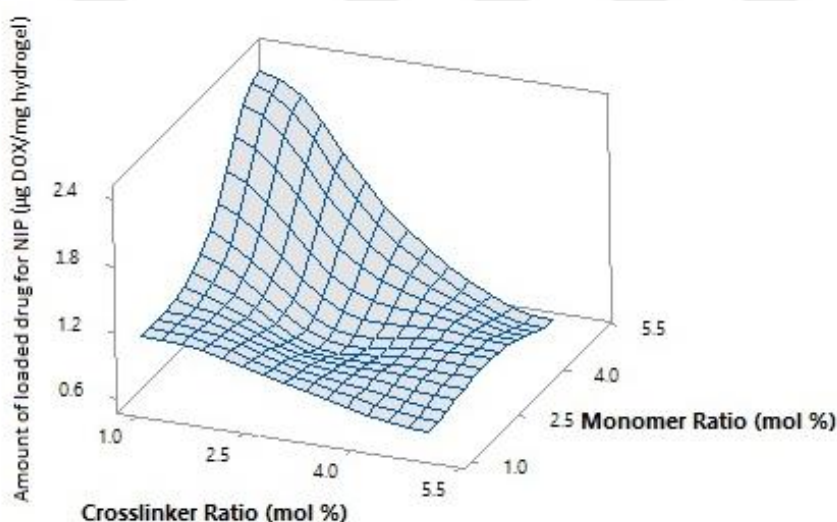


Figure 5.36 : Effect of crosslinker and monomer ratio on AA-EGDMA based non-imprinted hydrogels.

In the drug loading studies, DOX loading occurred in two different paths: specifically absorbed by AA functional groups or diffused in the hydrogel by swelling. Although, the swelling ratios were comparable between imprinted and non-imprinted counterparts, AA-EGDMA based imprinted hydrogels absorbed more drug than the non-imprinted counterparts (Figure 5.34). This was attributed to the amount of DOX absorbed specifically into the hydrogel by AA functional groups which became dominant compared with diffusion into the hydrogel by swelling. The affinity of the binding sites were the dominant factor in loading pattern of AA-EGDMA based hydrogels. It was clear that the imprinted hydrogels fabricated with AA and EGDMA, also had proper drug cavities with high imprinting factor.

In non-imprinted hydrogels, AA functional groups randomly distributed in the hydrogel matrix. It was suggested that crosslinker may prevent the interaction between AA and DOX in this hydrogels. Therefore, imprinted hydrogels absorbed more DOX than non-imprinted counterparts. For efficient imprinting the required crosslinker ratio was found as 3 % by mol. Over this ratio, both imprinted and non-imprinted hydrogel were intensely influenced by excess crosslinker ratio. In the preliminary studies the interaction capability of AA, IA, EGDMA and TEGDMA with DOX had been explored. As explained in the experimental part (Section 5.2.1), it was observed that the EGDMA, as crosslinker, interacted with doxycycline in the pre-polymerization step. This results revealed that the intensity of the interaction changed with crosslinker ratio in the pre-polymerization mixture.

The response surface diagram for IF values were presented in Figure 5.38 a-c. When the maximum points from the diagrams were collected M:X =3:3, M:T=3:0.205 X:T=3:0.205 were obtained. The estimated maximum ratio from the response surface was 3:3:0.205 (X:M:T). Moreover, the minimum ratio was obtained as 5:1:0.160(X:M:T).

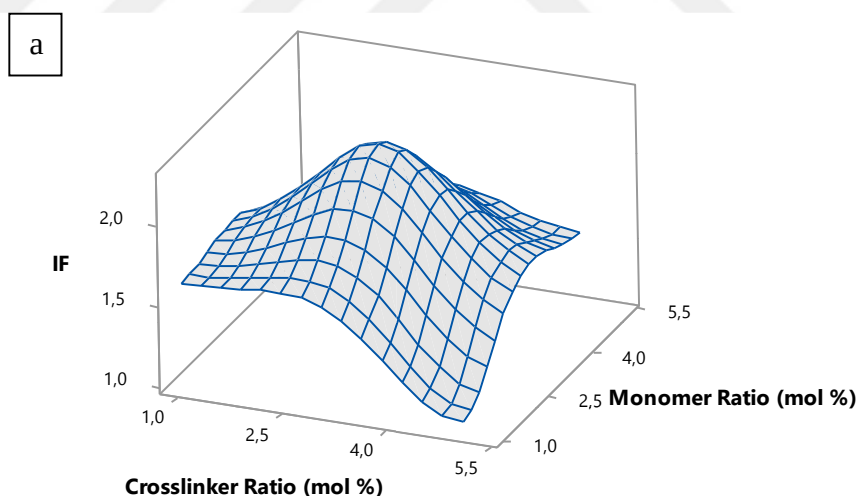


Figure 5.37 : 3D surface plots for IF values in AA-EGDMA hydrogels a. M:X effect b. M:T effect and c. X:T effect.

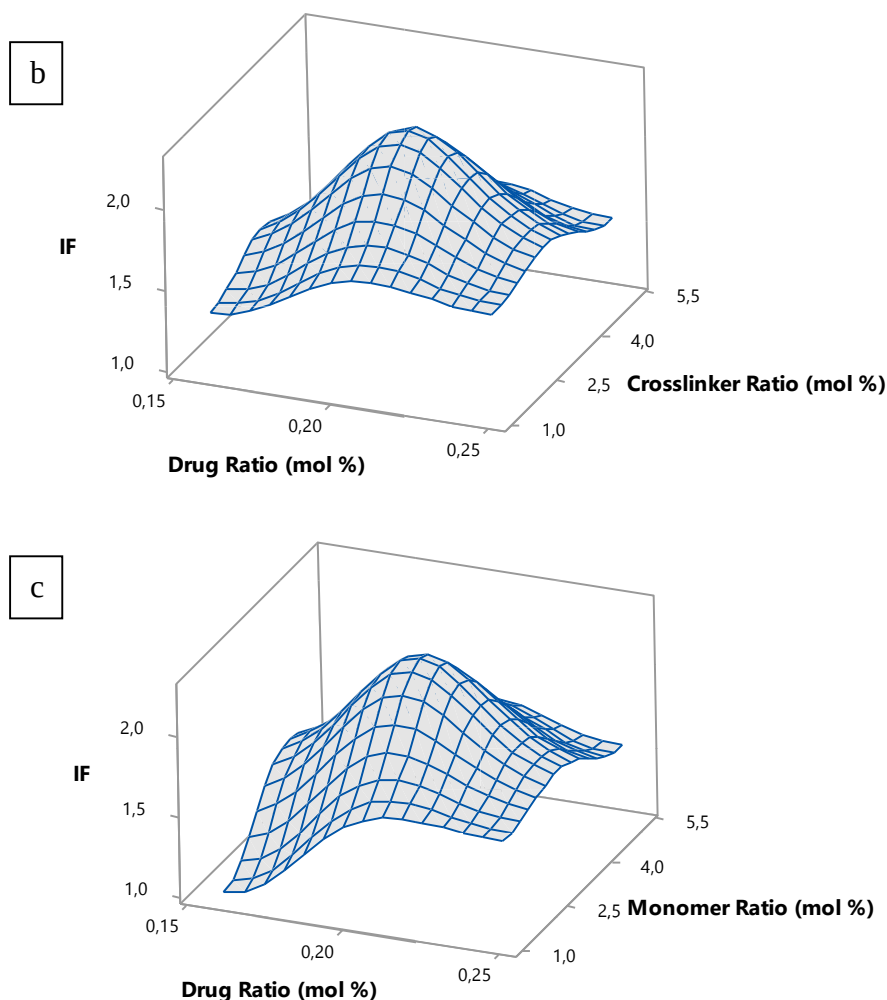


Figure 5.37 (Continued): 3D surface plots for IF values in AA-EGDMA hydrogels a. M:X effect b. M:T effect and c. X:T effect.

It can be said that adding adequate amount of monomer to polymerization mixture maximized interaction with DOX by increasing the number of non-covalent interactions between template and monomer. It is noteworthy to state that IF value decreased with large excess of monomer because of AA-AA and AA-EGDMA interactions which increased via large excess of functional monomer. Low amount of crosslinker led to the elimination of imprinting effect and decreased IF value. Moreover, optimum amount of crosslinker led to an increase in monomer-template interactions and formation of more binding cavities in the AA-EGDMA based imprinted hydrogels and increase of IF value. Excess amount of EGDMA made difficult for template to enter into the cavities and also might increase AA-EGDMA and DOX-EGDMA interactions and consequently, decreased both amount of loaded drug and IF value.

In the literature, there are many studies showing the influence of monomer ratio and M:T ratio on imprinting efficiency. Tom and coworkers indicated that increasing the amount of monomer with low amount of crosslinker in the polymerization mixture could enhance the imprinting efficiency [70]. Yanez et al., indicated that main function of monomers is to afford multiple binding sites with template molecule and also M:T ratio was significantly important in effective imprinting [137]. Bryne and coworker showed that if the M:T ratio was very small, there were not strong interaction between monomer and template molecule, resulting in no recognition [243]. According to Lorenzo et al., if adequate monomer ratio was not used to create binding sites, imprinting efficiency would be low [25]. Our results are in good agreement with these studies published in the literature.

Loading capacities of AA-TEGDMA based hydrogels were summarized in Table 5.33 and Figure 5.34. The results for the hydrogels synthesized with AA-TEGDMA (Hydrogel 11-15) and with AA-EGDMA (Hydrogel 1-5) indicated that loading capacities of AA-TEGDMA based hydrogels were lower than AA-EGDMA based hydrogels. The difference between AA-TEGDMA and AA-EGDMA hydrogels was pronounced by applying the imprinting. The loading capacity difference between non-imprinted counterparts became smaller and IF values decreased when TEGDMA was used as crosslinker. Although when the crosslinker ratio was lowest (1 mol %), AA-TEGDMA based imprinted hydrogels (hydrogel 11 and 12) absorbed more water than non-imprinted counterparts. Furthermore, the drug loading capacity was not higher than non-imprinted counterparts. The similar result was observed for AA-EGDMA based both MIPs and NIPs (hydrogel 1 and 2). However, AA-EGDMA based non-imprinted hydrogels had lower drug loading capacity significantly than AA-EGDMA based low crosslinked imprinted counterparts. It can be said that TEGDMA led to a reduction in diffusion amount of the DOX molecules into the hydrogels for both NIPs and MIPs. It may also be as a result of containing lower mesh size and higher $V_{2,s}$ than AA-EGDMA counterparts (Figure 5.32 and 5.31). The insufficient amount of crosslinker resulted in the formation of low number of the imprinted cavities for imprinted AA-TEGDMA based hydrogels. Another possibility was that when TEGDMA was used as crosslinker more monomers can stuck in the cavities compared to AA-EGDMA based hydrogels. In this case the cavities had a high affinity and the binding sites had an effective interaction with the drug molecule.

From Table 5.33, it was obtained that the AA-TEGDMA based MIPs had the ability of doxycycline hyclate loading slightly higher than non-imprinted counterparts. Imprinted hydrogel loaded 1.05 $\mu\text{g}/\text{mg}$ hydrogel and the non-imprinted bind on average 0.93 $\mu\text{g}/\text{mg}$ hydrogel for hydrogel 11 (IF = 1.14). Increasing 5-fold the proportion of TEGDMA was lowered the loading capacity down from 0.93 to 0.53 $\mu\text{g}/\text{mg}$ for non-imprinted ones (IF = 1.25). A decline was also observed for imprinted counterpart (from 1.05 to 0.68 $\mu\text{g}/\text{mg}$ hydrogel) for hydrogel 14. The monomer to template ratio (M:T ratio) changed from 6.25 to 4. Consequently, increasing the crosslinker ratio resulted in a decrease in loading capacity but this case increased imprinting efficiency by increasing IF value. Increasing 5-fold the proportion of AA raised slightly the loading capacity up to 0.61 $\mu\text{g}/\text{mg}$ hydrogel for non-imprinted hydrogel and also enhanced significantly the loading capacity of the imprinted hydrogel from 0.68 to 0.91 $\mu\text{g}/\text{mg}$ hydrogel (hydrogel 13, IF=1.58). Both IF value and loading capacities were went up. Moreover, reducing TEGDMA proportion 5-fold (hydrogel 12) instantaneously increased 3-folds loading capacity (1.89 $\mu\text{g}/\text{mg}$ hydrogel) of imprinted hydrogel and 4-folds of the non-imprinted counterpart (2.23 $\mu\text{g}/\text{mg}$ hydrogel) and IF value went down below 1 (hydrogel 12, IF=0.85). The M:T ratio for this hydrogel was equal to 20. Decreasing AA mol ratio to 3 % and increasing crosslinker ratio 3 % by mol again enhanced loading capacity for both imprinted and non-imprinted hydrogels (hydrogel 15) and increased IF value (IF=1.22). Briefly, IF values were minimum when the ratio of TEGDMA was 1 mol %. On the other hand, copolymerization of AA with high proportion of TEGDMA led to an increase in the IF values while loading capacities decreased for both MIP and NIP as expected.

As it can be seen from Figure 5.38 and 5.39, both AA-TEGDMA based imprinted and non-imprinted hydrogels had high DOX loading capacity when high functional monomer ratio and low crosslinker ratio was employed in polymerization. At this point (5 mol % AA and 1 mol % TEGDMA) non-imprinted hydrogel absorbed more drug than imprinted one (hydrogel 12). At high crosslinking ratio, loading capacities reduced drastically in both imprinted and non-imprinted hydrogels. But, the amount of decrease in loading capacity was less in the imprinted polymers (hydrogel 13). This proved that imprinting effect changed the loading abilities of the hydrogels.

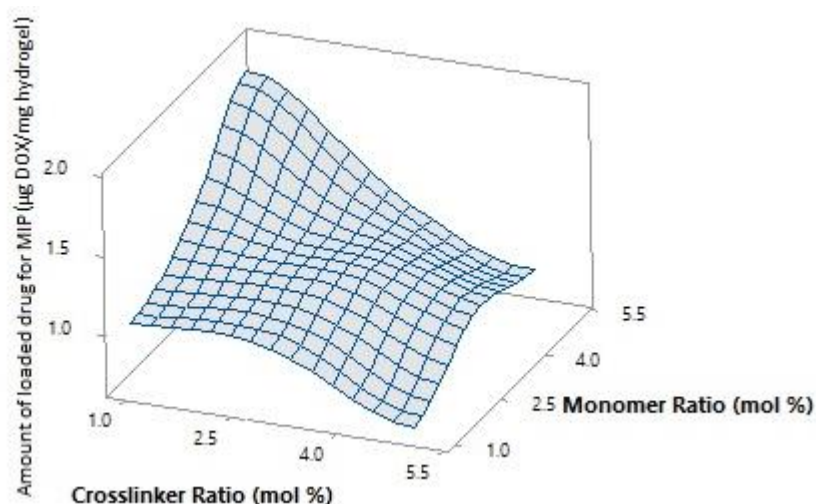


Figure 5.38 : 3D surface plot for crosslinker ratio monomer ratio interaction in AA-TEGDMA based imprinted hydrogels.

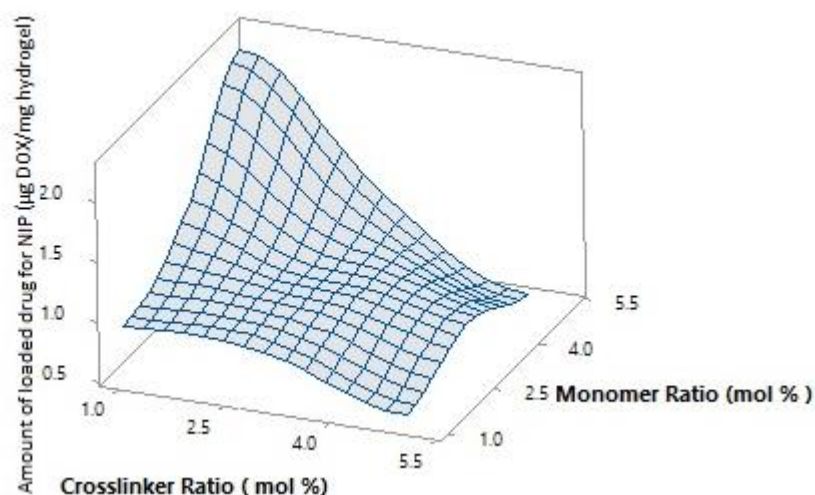


Figure 5.39 : 3D surface plot for crosslinker ratio monomer ratio interaction in AA-TEGDMA based non-imprinted hydrogels.

Figure 5.40 presented the response surface diagram for IF values (davies). When we compared AA-TEGDMA low crosslinked imprinted hydrogels with AA-TEGDMA non-imprinted counterparts, imprinted hydrogel 12 exhibited larger mesh size than non-imprinted ones and also had lowest IF value (IF=0.85). Imprinted hydrogel 11 had comparable mesh size with non-imprinted one and IF value slightly higher than 1 (IF=1.14)

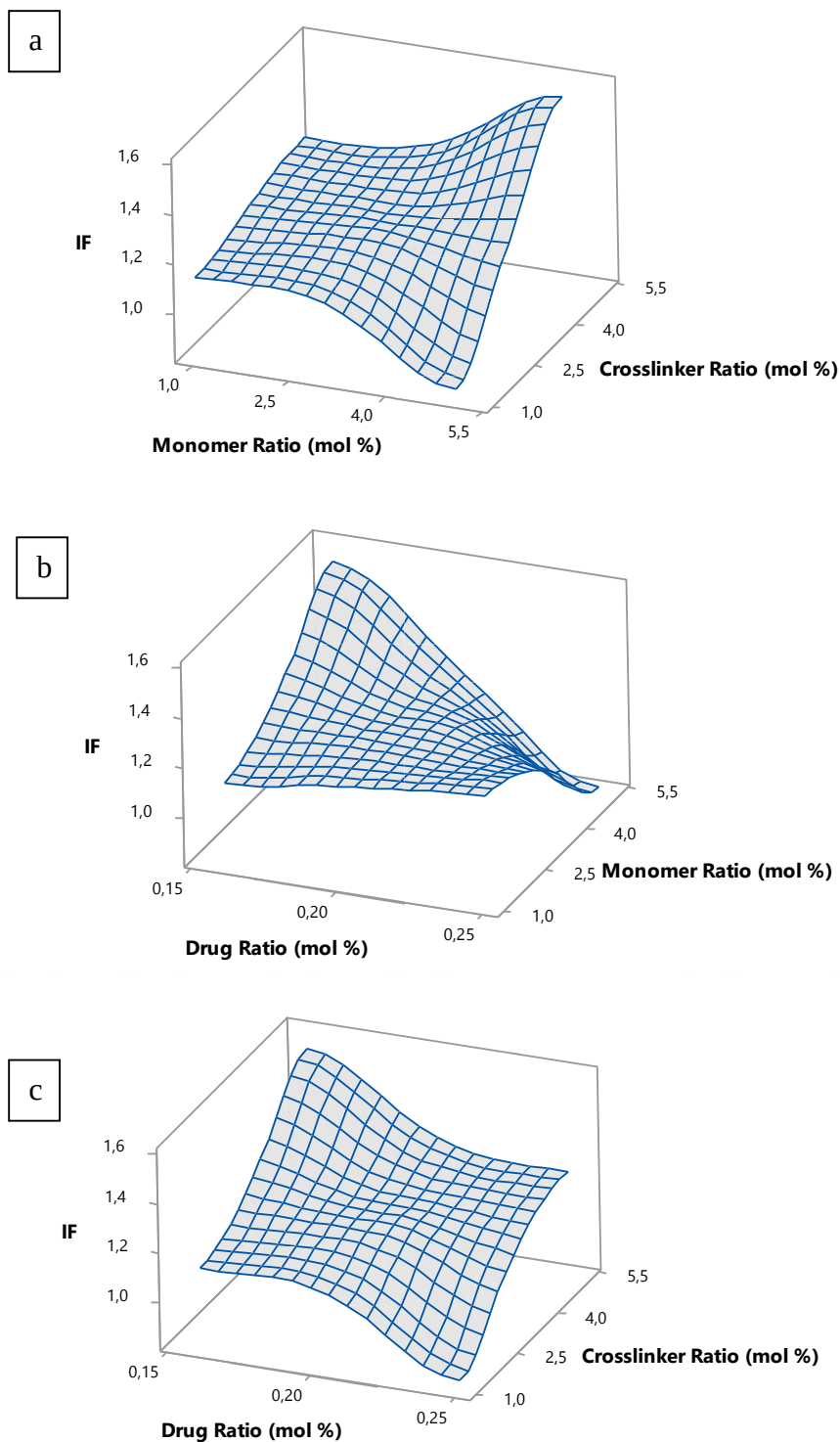


Figure 5.40 : IF values for AA-TEGDMA hydrogels a. M:X effect b. M:T effect and c. X:T effect.

The incorporation of low amount of TEGDMA with AA might not provide cavity stability to hold drug in the imprinted cavity. Crosslinker may not fully capture the drug molecule leading to a weak monomer template interaction despite the presence of monomer in the cavity. This reduced the loading difference between imprinted and

non-imprinted polymers (this case decreased IF value). When crosslinker ratio increased up to five fold, the mesh size fell down nearly to half. This reduction in the mesh size led to decrease in the loading capacity by hindering the entrance of drug molecules into the specified cavities. On the other hand, the mesh size reduction led to remain close AA monomers to each other and interacted with the drug molecule at multiple points effectively and may enhance cavity stability (hydrogel 14). The amount of monomer in the specified cavities increased to sufficient amount by increasing proportion of the monomer, and the imprinting effect became more obvious (hydrogel 13).

The maximum IF value was obtained once M:X:T was equal to 5:5:0.160. In another word, M:T and X:T ratio of AA-TEGDMA based hydrogels became slightly greater than optimum M:T and X:T ratio of AA-EGDMA based hydrogels. When AA was employed with TEGDMA, more monomer and crosslinker were required for a succesful imprinting. This results can be explained by molecular structures of TEGDMA and EGDMA and their interactions with DOX. EGDMA has two carbonyl (C=O) groups and two ether (–O–) groups while TEGDMA has four ether groups and two carbonyl groups. It is known from the literature [167,204,217] that template molecule can interact with crosslinker and surrounded by both monomer and crosslinker proportional to their binding ability to DOX. It was believed that some parts of the drug were captured by TEGDMA functional groups. Because of its slightly lower interaction with drug than EGDMA at high concentrations, the amount of monomer required for a successful imprinting may be increased. Since a portion of DOX was not captured by TEGDMA at high TEGDMA ratio, more AA was needed in the cavity. Although TEGDMA had a larger number of functional group, all these groups might not provide the strong interaction with the DOX. The absorbance titration studies also supported the proposed interpretation. High amount of drug in the polymerization may led to enhance of AA-DOX interaction. And this case did not led to prevention of succesful imprinting.

There are few studies in the literature detailing the effect of the nature of the crosslinker on polymer binding performance. In a study Sohavi and coworkers has used TRIM and EGDMA as cross-linkers, MAA as functional monomer and S-propranolol as template molecule [217]. Their results indicated that, although TRIM had more functional groups than EGDMA, it was found that each group did not interact with

template and monomer in the same proportion. In another study Hsu et al. explored the imprinting of Ribonucleas A into the hydrogel [244]. A crosslinker with four different of ethylene glycol (EGDMA, TEGDMA, PEG400DMA, PEG600DMA) was used. They indicated that PEG itself was often used to inhibit undesirable molecular binding. Their results showed that EGDMA and TEGDMA with short ethylene glycol chain were insufficient due to prevention of both the undesirable and desirable interactions. In agreement with our findings, these studies have reported that the relatively longer chain crosslinker reduces non-specific binding when used together with a functional monomer.

IA based hydrogels were synthesized with EGDMA and TEGDMA crosslinkers in the presence and the absence of template and results were summarized in Table 5.33. The results indicated that IF values were mostly below 1, except for hydrogel 10 (IF=1.16) for IA-EGDMA based hydrogels. The employment of high proportion of IA (5 mol %) with high proportion of EGDMA (5 mol % ratio) led to an increase in the IF value while loading capacity remained low for MIP loaded 1.13 $\mu\text{g}/\text{mg}$ hydrogel and NIP loaded 0.97 $\mu\text{g}/\text{mg}$ hydrogel (hydrogel 10). Decreasing 5-fold the amount of EGDMA increased the loading capacity from 0.97 to 3.79 $\mu\text{g}/\text{mg}$ hydrogel for non-imprinted (IF = 0.75), and also an increase was observed for imprinted counterpart (from 1.13 to 2.62 $\mu\text{g}/\text{mg}$ hydrogel) for hydrogel 8. The monomer:template ratio (M:T ratio) changed from 31.25 to 20. Consequently, decreasing crosslinker ratio led to an increase in the loading capacity and a reduction in IF value. Decreasing 5-fold the amount of IA dramatically reduced the loading capacity to 2.45 $\mu\text{g}/\text{mg}$ hydrogel for non-imprinted hydrogel and also decreased significantly the loading capacity of the imprinted hydrogel from 2.62 to 1.84 $\mu\text{g}/\text{mg}$ hydrogel (hydrogel 6, IF=0.87). As it can be seen for this hydrogel, loading capacities of both imprinted and non-imprinted hydrogels reduced but IF value went up. For hydrogel 7 increasing EGDMA proportion up to 5-fold decreased 3-folds the imprinted hydrogel loading capacity (0.68 $\mu\text{g}/\text{mg}$ hydrogel) and 3-folds the non-imprinted counterpart (0.84 $\mu\text{g}/\text{mg}$ hydrogel) and IF value went down below 1 (hydrogel 7, IF=0.82). The M:T ratio for this hydrogel was equal to 4. Increasing IA mol ratio to 3% and reducing crosslinker ratio 3% enhanced loading ability for non-imprinted hydrogel however decreased for imprinted ones (hydrogel 9) and also increased IF value (IF=0.68).

By interpreting the results given in Figure 5.41 and 5.42, it can be realized that IA-EGDMA based imprinted hydrogels loaded lower amount of drug than non-imprinted ones at higher monomer ratio. On the other hand, the loading capacity with a higher crosslinker ratio varied for both NIP and MIP with the same trend except for hydrogel 9 and hydrogel 10. At mid-point (3 mol % IA and 3 mol % EGDMA) non-imprinted hydrogel absorbed greater amount of DOX than imprinted one (hydrogel 9). When 5 mol % crosslinker and 5 mol % functional monomer was employed, imprinted hydrogel loaded higher amount of drug than non-imprinted hydrogel (IF value was higher than 1), loading capacities reduced significantly in both imprinted and non-imprinted hydrogels (hydrogel 10).

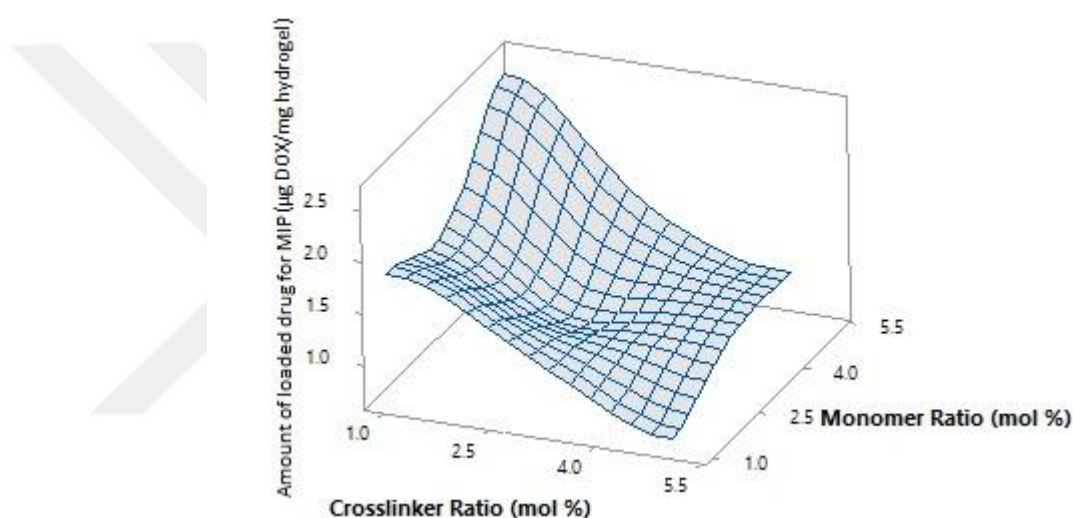


Figure 5.41 : 3D surface plot for crosslinker ratio monomer ratio interaction in IA-EGDMA imprinted hydrogels.

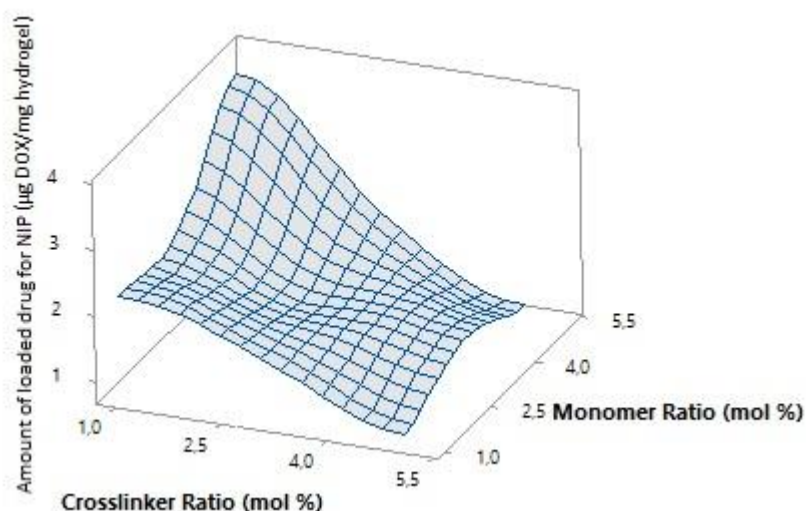


Figure 5.42 : 3D surface plot for crosslinker ratio monomer ratio interaction in IA-EGDMA non-imprinted hydrogels.

As it can be seen from Figure 5.43 a-b, two of the hydrogels had larger M:T value (M:T ratio for imprinted hydrogel 10 was equal to 31.25 and for imprinted hydrogel 8 was equal to 20) and the IF value increased slightly above 1, as M:T increased from 20 to 31.25 .

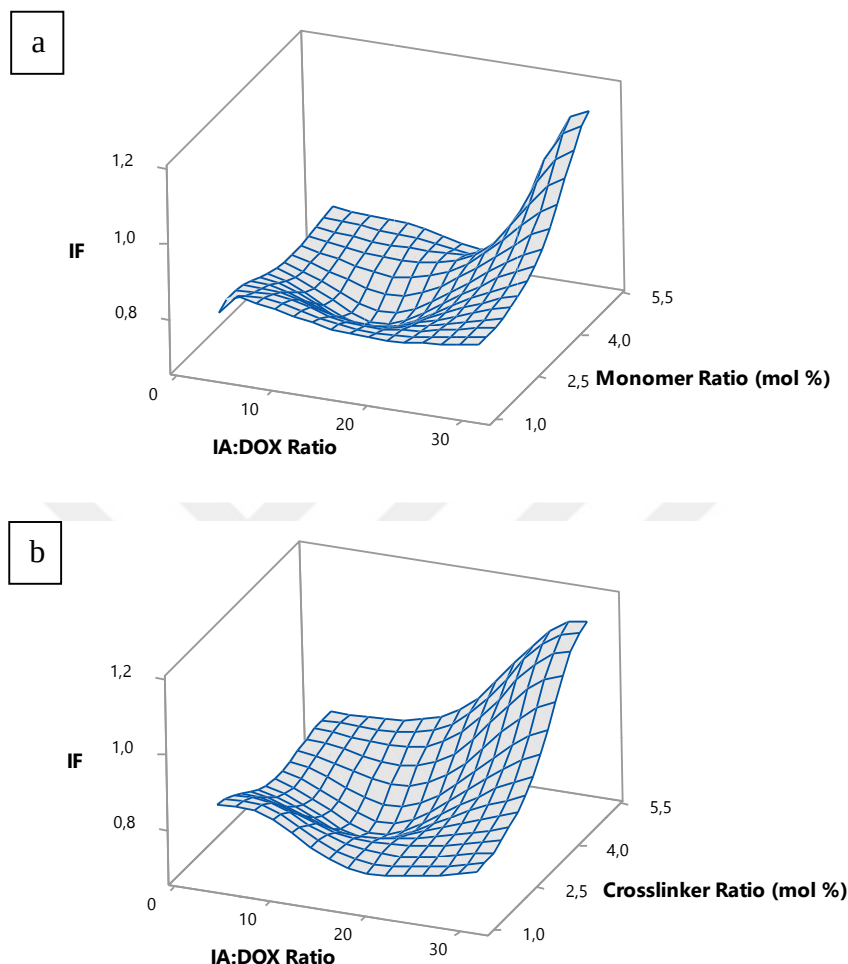


Figure 5.43 : 3D surface plots for IF values in IA-EGDMA based hydrogels a. M:T effect with monomer ratio b. M:T effect with crosslinker ratio.

The lowest crosslinker ratio led to an increase in drug binding ability of the hydrogels because of relatively large mesh size and high swelling ratio (hydrogel 6 and 8). Increasing the crosslinker ratio resulted in the shrinkage of mesh size of the cavities and prevention of the drug molecules absorption to the binding sites of the cavities due to reducing water absorption of the hydrogel for hydrogels.

The results for AA-EGDMA and IA-EGDMA based hydrogels showed that replacement of AA by IA led to an improvement in the loading capacity of the non-imprinted hydrogels. This was very likely the result of itaconic acid which can form stronger interaction with DOX molecules than acrylic acid. This was also a good

indication that IA itself had a high binding affinity to DOX. As it can be seen in Table 4.1 unlike AA which contains one C=O and –O–, IA includes two C=O and two –O– groups, thus, would be expected to better fit and interaction with DOX in the binding cavity. The preliminary results also demonstrated that IA had highest affinity to apply to the molecular imprinting hydrogels.

Furthermore, increasing the IA ratio may be led to randomly distribution of the monomers around the binding sites over the saturation point. During complexation, there may be competition of the IA and EGDMA due to interaction with DOX. When some parts of the DOX were captured by EGDMA, IA which had very high binding affinity to DOX can be inclined to bind DOX. In this case, the imprinting performance can be reduced because of IA's non-specific binding demand. On the other hand, it was possible that, DOX and IA interacted with more number of binding sites which may cause a decrease in number of imprinted cavities but also led to a decrease in the number of randomly distributed IA monomer around the hydrogels matrix. Furthermore, non-specific DOX-EGDMA interactions were eliminated by using monomer which had higher affinity to the template. It can be said that most of the drug molecule strongly interacted with IA monomers and small number of them interacted with the other hydrogel components.

In literature, it is reported that template can interact with other hydrogel components according to their affinities to template [217]. The number of functional monomers gathered to form the binding site increased with enhancing binding affinity of monomer to the template [25,100]. In another study, Chomon et al. studied with AAm (acrylamid) and BzMA (benzylmethacrylate (BzMA)) as functional monomers [245]. Their results proved that if a monomer had higher interaction ability to template, the number of binding sites may reduce because of different arrangement around the template. They suggested that when BzMA improved the loading capacity of the non-imprinted hydrogels, the more number of BzMA localization in the imprinted cavities caused reduction in the number of binding sites with respect to AAm based hydrogels. They also demonstrated that less number of imprinted cavities were created but they had high affinity to BzMA. In another study, Inan investigated the monomer, crosslinker and template interactions using computational simulation. She indicated that monomers and crosslinkers competed with each other to bind template molecule. This competition caused to decrease amount of loaded drug on the hydrogels [167].

Our results are in good agreement with the results published by above-mentioned researchers.

IA-TEGDMA based hydrogels were investigated and the results can be seen in Table 5.33. These results demonstrated that IA-TEGDMA hydrogels loaded greater amount of DOX than AA-TEGDMA hydrogels but loaded lower amount of DOX than IA-EGDMA hydrogels for both MIPs and NIPs. The IA-TEGDMA based MIPs were capable of binding a slightly lower degree of doxycycline hyclate with respect to non-imprinted counterparts (Figure 5.34). Specifically, imprinted hydrogel absorbed 2.37 $\mu\text{g}/\text{mg}$ hydrogel and the non-imprinted absorbed 3.13 $\mu\text{g}/\text{mg}$ hydrogel for hydrogel 19 (IF =0.76). This is the lowest IF value for IA-TEGDMA based hydrogels. Increasing 5-fold the proportion of TEGDMA decreased the loading capacity drastically from 3.13 to 1.20 $\mu\text{g}/\text{mg}$ for non-imprinted (IF = 1.13), and also significant differences were observed for imprinted counterpart (from 2.37 to 1.35 $\mu\text{g}/\text{mg}$ hydrogel) for hydrogel 17. In this hydrogel, the monomer:template ratio (M:T ratio) changed from 31.25 to 20. In this condition, increasing the crosslinker ratio led to a decrease in loading capacity however imprinting efficiency increased by increasing IF. Reducing 5-fold the amount of IA reduced the quantity of loaded DOX from 1.20 to 0.77 $\mu\text{g}/\text{mg}$ hydrogel for non-imprinted hydrogel and also reduced the amount of loaded DOX of the imprinted hydrogel from 1.35 to 0.76 $\mu\text{g}/\text{mg}$ hydrogel (hydrogel 18 IF=0.99). Both IF values and loading capacities demonstrated downfall trend. In hydrogel 16, a 5-fold decrease in IA amount caused a two-fold enhancement in loading capacity of both imprinted and non-imprinted hydrogel (IF=0.98). The M:T ratio for this hydrogel was set to 4. For hydrogel 20, when both the crosslinker and monomer ratio increased, loading capacity was slightly decreased. However, IF value did not experience any change.

As it can be seen in Figures 5.44 and 5.45, it is observed that when crosslinker ratio increased, both IA-TEGDMA based imprinted and non-imprinted hydrogels had low DOX loading capacity. When high ratio of functional monomer employed, high loading capacity was enhanced. By applying maximum values for all components (5 mol % IA, 5 mol % EGDMA and 0.250 mol % DOX), non-imprinted hydrogel absorbed lower amount of DOX than imprinted one (hydrogel 17). Moreover, when 1% crosslinker, 5% functional monomer and 0.160 mol % DOX was used, loading capacities enhanced remarkably in both imprinted and non-imprinted hydrogels

(hydrogel 19). Non-imprinted hydrogels loaded greater or equal amount of drug than imprinted hydrogel.

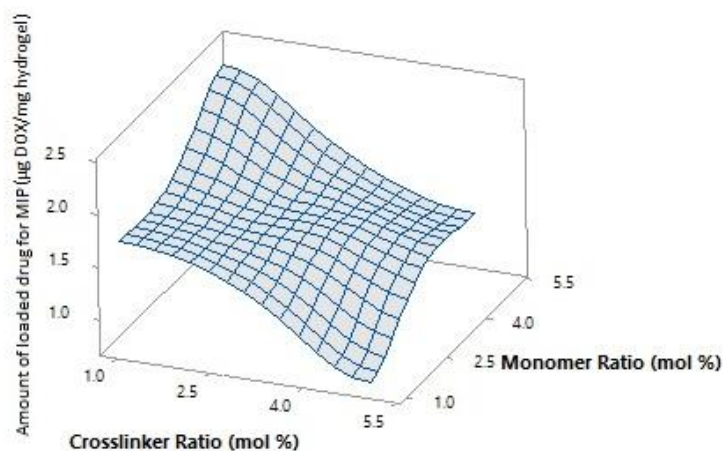


Figure 5.44 : 3D surface plot for crosslinker ratio monomer ratio interaction in IA-TEGDMA imprinted hydrogels.

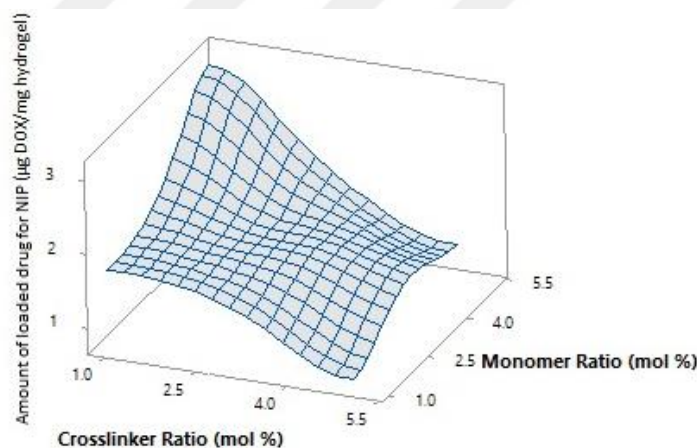


Figure 5.45 : 3D surface plot for crosslinker ratio monomer ratio interaction in IA-TEGDMA non-imprinted hydrogels.

This can be described by the cavities number/size and loading capacity features of the hydrogels. The number and size of the TEGDMA based MIPs cavities were different from EGDMA based MIPs. IA-TEGDMA based imprinted hydrogels had a tighter structure than non-imprinted counterparts. Therefore, mesh size of the MIPs were smaller than NIPs (Figure 5.33b) and also $V_{2,s}$ values of MIPs were greater than NIPs in the loading solution (Figure 5.31a) of IA-TEGDMA based hydrogels. It was believed that structural differences originated from the arrangement of monomers and crosslinkers around the template molecule during pre-polymerization as mentioned in structural analysis section. The employment of a larger monomer with TEGDMA led to a fully coverage of the drug molecule by functional monomer and the desired

interaction due to the presence of monomers in the cavity was achieved. Consequently, functional monomer size should increase corresponding to the increase in crosslinker length.

The number of monomer located in the cavities specifically may be great due to the high affinity of IA to DOX. As known from the preliminary studies, DOX can interact with TEGDMA as well as IA. Therefore, non-imprinted hydrogels loaded with slightly greater amount of DOX and also the loaded drugs in hydrogel matrix bind to non-specific binding sites because of the higher affinity of IA itself to DOX.

Figure 5.46 presented the response surface diagram for IF values of containing IA-TEGDMA hydrogels. The loading trend was different from IA-EGDMA based hydrogels as a function of IA:DOX ratio, if the IF values considered (Figure 5.46). It mean that the M:T ratio, the individual added drug, monomer and crosslinker ratios played crucial role on drug loading performance. When the drug ratio existed in the synthesis was low, IF value decreased. In this case, the used monomer ratio was too high compared to the template molecules in the reaction medium. Since insufficient number of cavities were formed, the number of randomly distributed monomer rised. Using both the IA:DOX and the monomer ratio at highest ratios lowered the IF value which was similar to the first case. Employment of excess number of monomer resulted in the formation of non-specific binding sites. However, when the crosslinker ratio and M:T ratio were used in higher ratios, the IF value increased. Therefore, the amount of crosslinker should be sufficient in order to the template and monomer to be able to come together enough to create a sufficient number of cavities. Increasing crosslinker ratio may allow to a better stability of DOX in the cavities. At this combination, monomer became close to the template in the cavity and the interaction may be stronger than the all other combinations. Additionally, when the monomer ratio was 1 mol %, there was no difference in the loading capacities between the imprinted and non-imprinted hydrogels. Monomer ratio of 1 mol % was not be sufficient for the formation of adequate number of binding site. The amount of monomer in the cavities may be low in order to make a difference between imprinted and non-imprinted hydrogels. On the other hand, mesh size was greater than the others in hydrogel with only 1% IA and 1% TEGDMA (hydrogel 16). Hence, increasing IA or TEGDMA ratio alone was enough to reduce the mesh size (Figure 5.33). As a result, it was difficult to determine the success of imprinting by considering solely the loading capacities and

IF values. This can be clearly understood when the efficiency of imprinting was compared with each other after releasing studies.

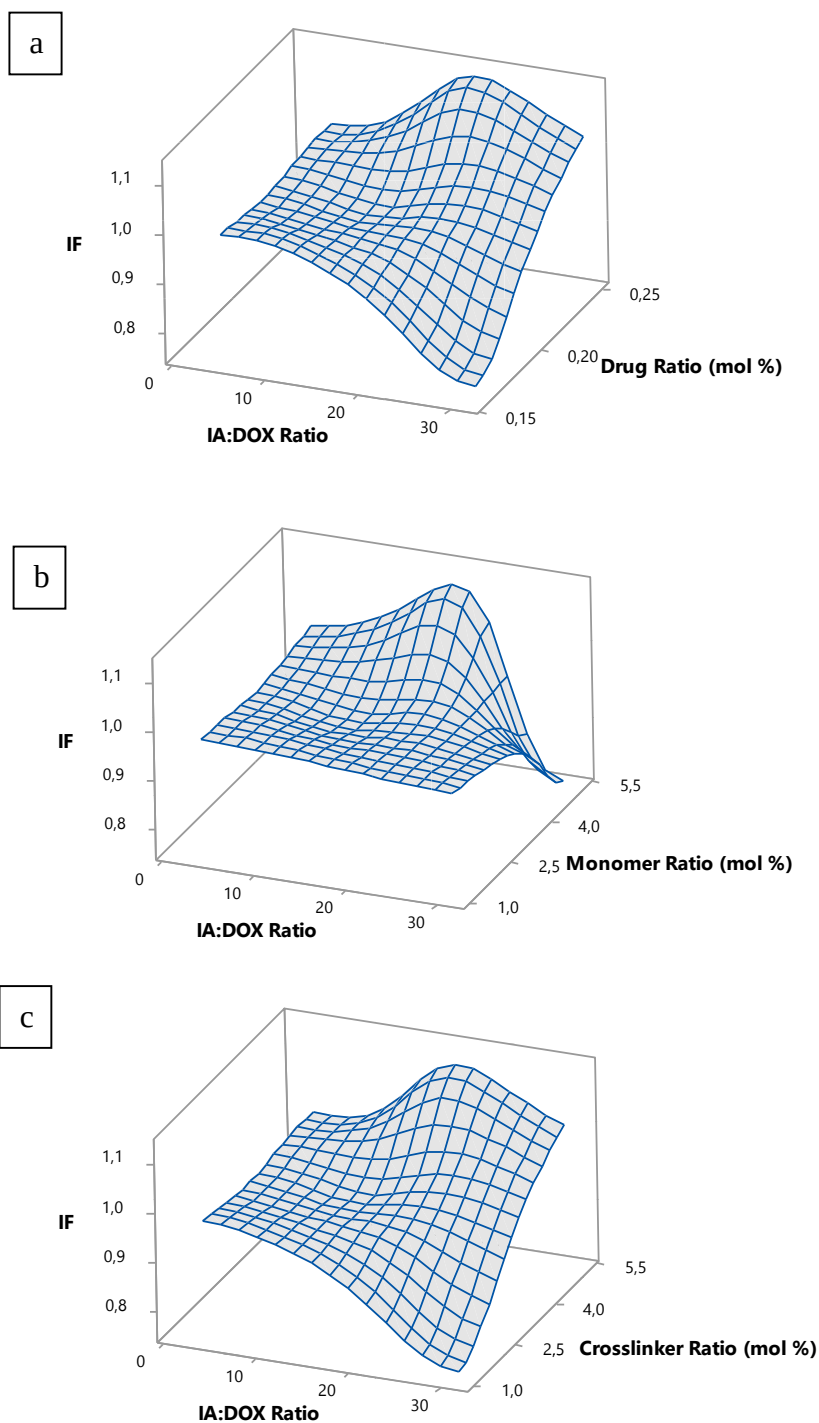


Figure 5.46 : 3D surface plots for IF values in IA-TEGDMA hydrogels a. M:T effect with drug ratio b. M:T effect with monomer ratio and c. M:T effect with crosslinker ratio.

5.3.4 Drug release studies

Section 5.1.2–5.3.2 of the study were dealing with the preparation, characterization and morphological structure of both molecular imprinted and non-imprinted hydrogels. Absorbance titration studies using UV-Vis spectroscopy were carried out in order to achieve a comprehensible understanding of interactions between the monomer-template and crosslinker-template. These studies could shed light on interaction intensity of the template with the monomers or crosslinkers in the pre-polymerization mixture which allow the formation of binding sites with different affinity levels. Some different cases have been developed for the resulting hydrogels, especially for the imprinted hydrogels prepared with different monomer-crosslinker combinations and ratios. The scope of this section was to clarify release behavior of the both non-imprinted and imprinted hydrogels which had different number of binding sites and affinity levels within the hydrogel matrix. The impact of the variables on the generation of drug release from the hydrogels was investigated as a function of time.

Release experiments were performed after hydrogels were loaded at a constant initial drug loading concentration (1 mM DOX solution). Subsequently, the hydrogels transferred to fresh release medium after 48 hours, drug release did not continue after 48 hours in 0.9 % NaCl solution.

5.3.4.1 Diffusion coefficients of the hydrogels

The effect of the network structure on diffusion coefficients for the drug release from all hydrogel formulations were investigated and were presented in Figure 5.47. In general, the imprinted networks had lower diffusion coefficients than non-imprinted ones. It may be concluded that the multiplicity and arrangement of functional groups are responsible for drug binding and obstruction in transport. The strength of the non-covalent specific interactions and crosslink structure also effected the controlled release of drug. These results indicated that the drug release can be accustomed with the hydrogel structure and imprinting process.

Figure 5.47 also indicated that, the diffusion coefficient decreased with nature of the crosslinker. It can be suggested that drug movement from one side to another became difficult because of the complex crosslink structure of TEGDMA based hydrogels. IA containing hydrogels had lower diffusion coefficient due to the stronger interaction

with DOX molecule (electrostatic and hydrogen bond). A considerable increase in DOX diffusion coefficient was observed as the amount of crosslinker increased. It may be attributed to the fact that available diffusion path for DOX diffusion was shorter due to the low volume fraction in swollen state in releasing medium. It can be also said that there was a weak drug-monomer interaction which caused to lower mass transfer resistance. The increase in crosslinker ratio declined swelling of the hydrogel and it might prevent the ionization of carboxyl groups. The drug in the release medium was in the form of zwitterion, and it may also interact with the functional groups of the monomers via electrostatic and hydrogen bonding. Electrostatic interaction which was a stronger interaction than hydrogen bonding, was reduced between monomers. The increase in the diffusion coefficient may be due to the shortening of the diffusion path as well as the weakening of the monomer drug interaction. Similar results were obtained by Syed Majid and coworker; diffusion coefficient high crosslink ratio caused to an increase in diffusion coefficient. In an another study, Mudassir and coworker prepared hydrogels with IA/MMA and EGDMA with different crosslinker ratios and found that diffusion coefficients increased with increasing the crosslinker ratio [179].

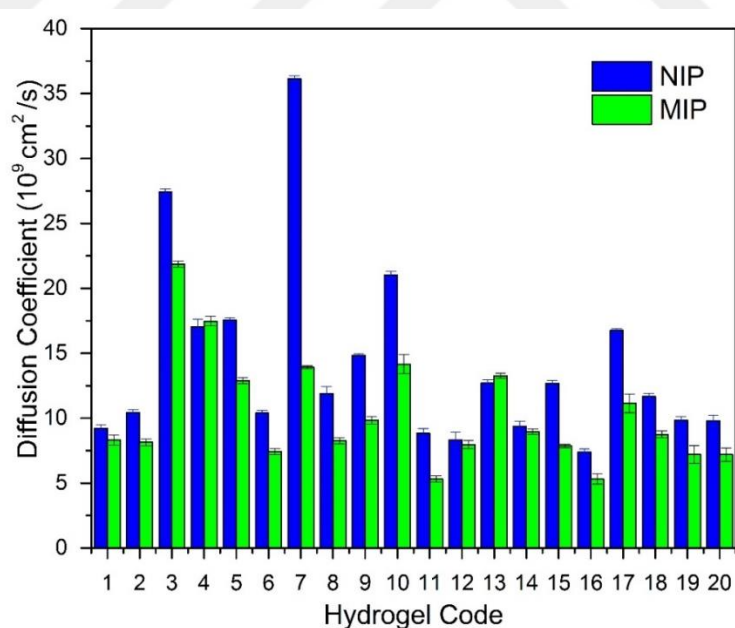


Figure 5.47 : Diffusion coefficients of the both MIPs and NIPs.

In the literature slower diffusion of drug molecules from imprinted network was explained via tumbling effect by Vankatesh et al. [172]. Figure 5.48 emphasized tumbling effect that revealed the movement of the drug through the hydrogel matrix

from one binding site to the next resulting in slower diffusion and prolonged releasing kinetic.

There were enhanced and more effective binding movements in an specifically imprinted network including multifunctional binding sites in the cavities than the non-imprinted network. Non-imprinted network included randomly distributed functional monomers. The non-imprinted network was not controlled in order to polymerize around the template during polymerization and hence had no specific memory site. Networks which were generated with single or randomly arranged functional monomers contain very small number of interaction point with the drug to bind to for an extended release. Due to this fact, diffusion coefficient of the imprinted matrix was lower than non-imprinted ones. Temporarily drug binding may cause to a prevention of free template transport. On the other hand, it was known that diffusion coefficient of an imprinted hydrogel can be affected by three factors: mesh size, template size and drug-monomer interaction (imprinting effect). The arrangement of the functional groups and crosslink structure in the imprinted cavities may lead to sustained release of the DOX.

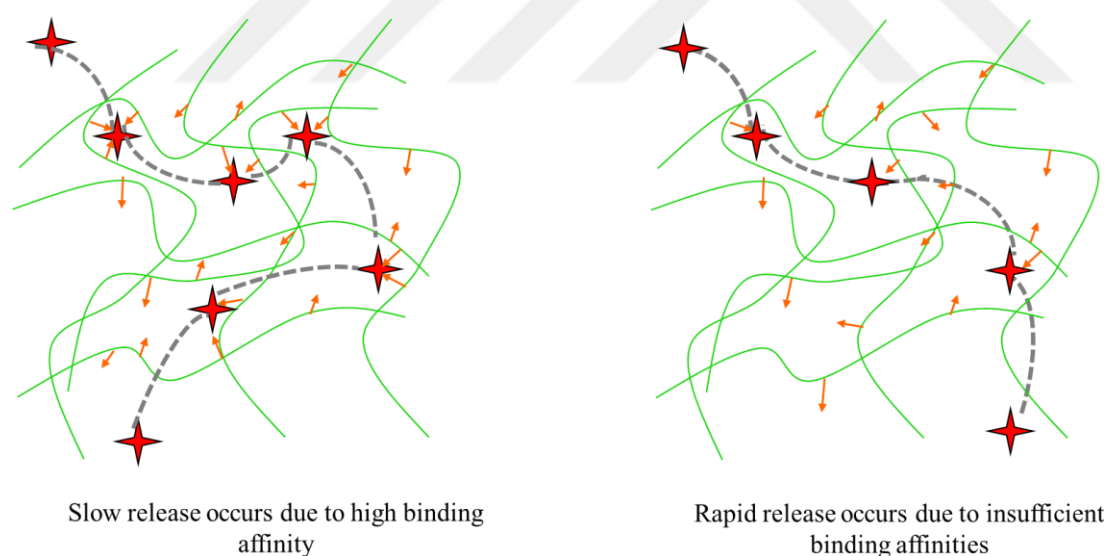


Figure 5.48 : Schematic representation of tumbling effect adapted from [172].

The release curves for 48 hours are presented in Figure 5.49 for both non-imprinted and imprinted hydrogels. There was a statistically significant difference ($p < 0.05$) between the total amount of the released drug for imprinted and non-imprinted hydrogels over 48 hours (Table 5.33). Not all but a large number of MIPs showed an

advantage by releasing a greater amount of DOX with more controllable feature than non-imprinted counterparts.

The results demonstrated that the different hydrogel contents had a noteworthy effect on the releasing behavior of the hydrogels for both NIPs and MIPs. Release profiles of 0.9 % NaCl solution were also influenced by the nature of crosslinkers and proportion of the functional monomer, crosslinker and template presence in the polymerization process.

Since the thesis subject was a wide experimental study, we conducted the cumulative DOX release studies on a series of hydrogels containing different monomer-crosslinker combinations in order to investigate imprinting effect. All the quantitative results were presented as μg DOX/mg dry hydrogel weight and written as μg only in the text.

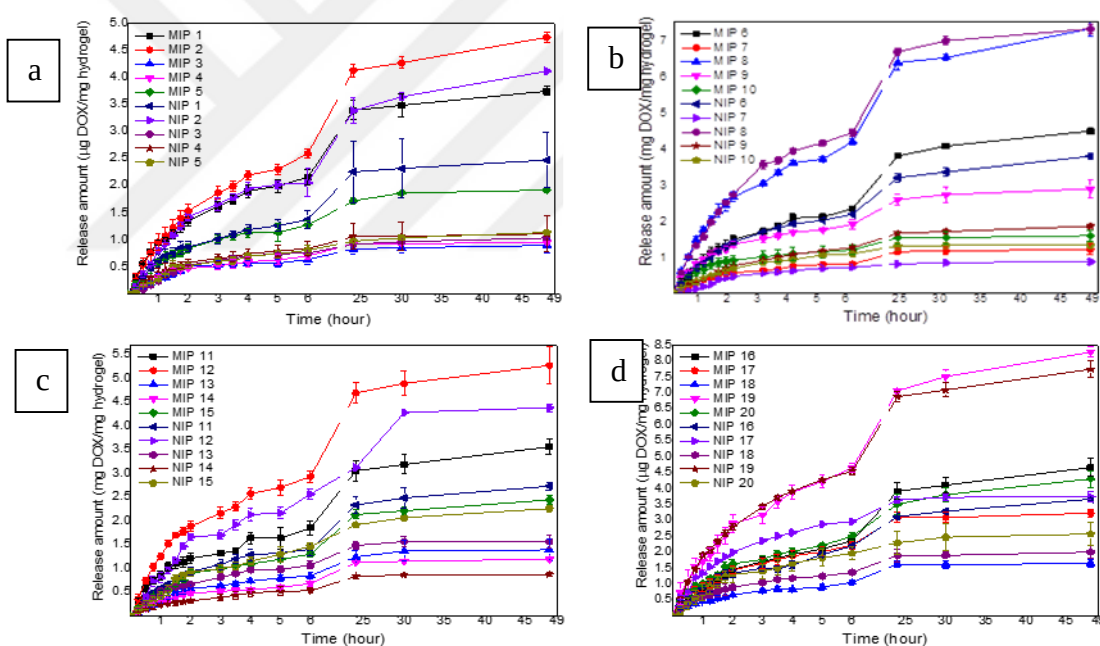


Figure 5.49 : Amount of DOX released in 0.9 % NaCl solution for imprinted and non-imprinted hydrogels.

Table 5.33 : Amount of DOX loaded and released from imprinted and non-imprinted hydrogels.

Hydrogel Code	Composition of polymerization mixture					MIP		NIP		IF
	Functional Monomer	F. Monomer Ratio (mol %)	Crosslinker	Crosslinker Ratio (mol %)	Drug Ratio (mol %)	Total Drug Released in 48 h.	Loaded Drug in 72 h.	Total Drug Released in 48 h.	Loaded Drug in 72 h.	
1	AA	1	EGDMA	1	0.25	3.75	1.81	2.47	1.12	1.62
2	AA	5	EGDMA	1	0.16	4.74	3.25	4.12	2.41	1.36
3	AA	1	EGDMA	5	0.16	0.90	0.69	1.04	0.68	1.03
4	AA	5	EGDMA	5	0.25	0.95	0.89	1.11	0.58	1.48
5	AA	3	EGDMA	3	0.205	1.92	1.55	1.14	0.69	2.25
6	IA	1	EGDMA	1	0.16	4.49	1.84	3.79	2.25	0.87
7	IA	1	EGDMA	5	0.25	1.21	0.68	0.87	0.84	0.82
8	IA	5	EGDMA	1	0.25	7.32	2.62	7.31	3.79	0.75
9	IA	3	EGDMA	3	0.205	2.89	1.02	1.86	1.52	0.68
10	IA	5	EGDMA	5	0.16	1.59	1.13	1.32	0.97	1.16
11	AA	1	TEGDMA	1	0.16	3.56	1.05	2.74	0.93	1.14
12	AA	5	TEGDMA	1	0.25	5.28	1.89	4.39	2.23	0.85
13	AA	5	TEGDMA	5	0.16	1.40	0.91	1.57	0.61	1.58
14	AA	1	TEGDMA	5	0.25	1.20	0.68	0.88	0.532	1.25
15	AA	3	TEGDMA	3	0.205	2.45	1.22	2.27	1.0	1.22
16	IA	1	TEGDMA	1	0.25	4.67	1.71	3.67	1.75	0.98
17	IA	5	TEGDMA	5	0.25	3.23	1.35	3.75	1.20	1.13
18	IA	1	TEGDMA	5	0.16	1.66	0.76	2.01	0.77	0.99
19	IA	5	TEGDMA	1	0.16	8.29	2.37	7.75	3.13	0.76
20	IA	3	TEGDMA	3	0.205	4.31	1.59	2.58	1.70	0.94

Firstly, AA based hydrogels have been discussed. Amount of released DOX from AA-EGDMA containing hydrogels were statistically different for $p < 0.05$ (Table 5.33). In Figure 5.50 and 5.51, we illustrated the relationship between the individual components and their interactions with each other. When monomer ratio incorporated in the hydrogel was increased, non-imprinted hydrogels released slightly higher amount of DOX than low monomer ratio counterparts (Figure 5.51). Additionally, AA:DOX ratio did not affect adequately releasing behavior at low monomer ratios (1 % and 3 % by mol). Using higher amount of monomer in the hydrogel network led to noticable increase of released DOX when AA:DOX ratio was equal to 31.25. When AA: DOX ratio was equal to 20 and monomer ratio was equal to 5 mol % (Hydrogel 4), the amount of released DOX fell to down to a lowest value.

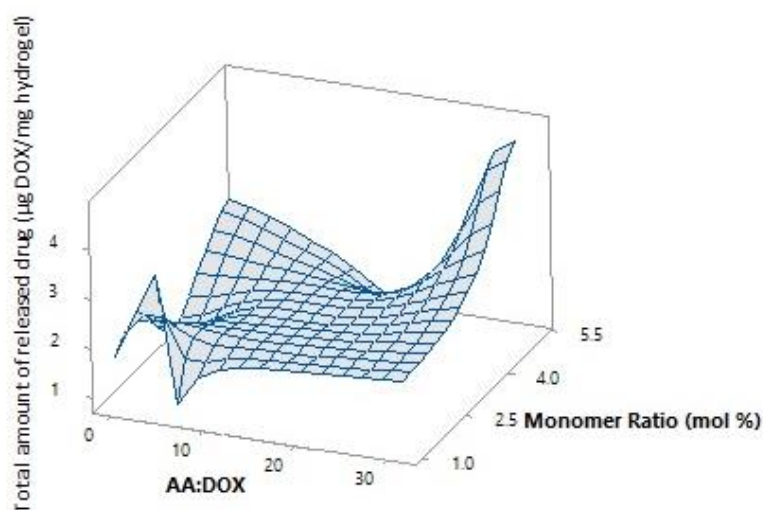


Figure 5.50 : 3D surface plot for monomer ratio AA:DOX ratio interaction in AA-EGDMA hydrogels .

We observed a clear trend that increasing crosslinker ratio in the hydrogels decreased the cumulative released mass of DOX for AA-EGDMA based hydrogels (Figure 5.51). However, the amount of reduction for released DOX for MIP hydrogels was more than NIP counterparts. Increasing AA proportion to 5 mol % with high crosslinker ratio did not change total amount of the released drug remarkably. Although IF value was higher than 1, imprinted hydrogel did not release more amount of drug than the non-imprinted counterpart (hydrogel 4). Reducing the crosslinker ratio to 1 mol % in the hydrogels increased cumulative released mass of DOX for both imprinted and non-imprinted hydrogels. However, the cumulative release increased two fold for non-imprinted hydrogels, and increased less than the two-fold for imprinted hydrogels. Up to this

point, the hydrogel had 5 mol % EGDMA, mesh size was small and the swelling of the hydrogel prevented by high crosslinker ratio. Adding high amount of AA into hydrogel structure might led to ionization of large number of carboxyl groups. It can be stated that deprotonation of acrylic acid increased the electrostatic interactions with DOX (in zwitter ion form with positively and negatively charged) and immobilized the DOX into the hydrogel because of excess amount of monomer. Another explanation is that, increasing the crosslinker ratio in the hydrogel decreased the amount of released DOX due to rigidity of the structure and prevention the motion of the drug.

It was detected that non-imprinted and imprinted hydrogels released lower amount of DOX with increasing crosslinker ratio from 1 mol % to 5 mol % (Figure 5.51). When the AA:DOX ratio exceeded 20, the amount of released DOX increased remarkably. While AA:DOX was equal to or lower than 20, the amount of increased DOX had comparable quantities. Multi-organized functional monomers around DOX intensified interactions with DOX molecules and showed significantly effect of imprinting. At high level crosslinker ratio (hydrogel 3 and 4), regardless of the AA:DOX ratio, the drug release was greater quantity for imprinted hydrogels than the non-imprinted ones. These hydrogels had high volume fraction in swollen state in the releasing medium. These structural difference was one of the reasons in lower DOX release when compared to the others. When the percentage difference in the amount of release between the imprinted and non-imprinted polymers is considered, it was observed clearly that imprinting efficiency was correlated with imprinting factor. The differences in the amount of releasing between imprinted and non-imprinted hydrogels reached to a maximum for hydrogel 5 with highest imprinting factor (68% greater amount of DOX released from imprinted one). This strengthened the claim that monomer, drug and crosslinker amount used to synthesize hydrogel contributed to the success of imprinting by changing structure properties and releasing pattern of the hydrogel.

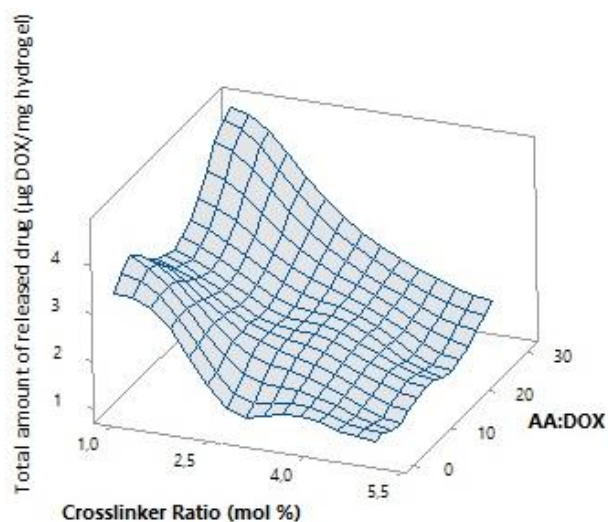


Figure 5.51 : 3D surface plot for crosslinker ratio AA:DOX ratio interaction in AA-EGDMA hydrogels.

Non-imprinted hydrogels containing AA-TEGDMA (hydrogels 11-15) exhibited a similar trend with AA-EGDMA based non-imprinted hydrogels. While crosslinker ratio increased, total amount of released DOX decreased. On the other hand, as we can see from Table 5.33 that AA-TEGDMA based hydrogels loaded fewer drugs than AA-EGDMA based hydrogels.

The amount of loaded and released drug for AA-TEGDMA based hydrogels was decreased and increased compared with AA-EGDMA based hydrogels, respectively. While interactions occurred between the DOX and each functional monomer individually, imprinted hydrogels containing AA-TEGDMA maximized the number of specific interaction with DOX. Mesh size calculations were indicated that shorter distance between functional groups and drug can be achievable by using TEGDMA. $V_{2,s}$ value differences between imprinted and non-imprinted hydrogels were low in loading solution and were large in releasing medium for AA-TEGDMA based hydrogels (Figure 5.31). The more strength interactions with DOX molecule may enhance affinity, decreased diffusion coefficient and also increased cumulative released drug. It means that not only structural properties influenced drug release behavior of the hydrogels but also functional monomer template interactions play a significant role in this regard.

It was observed that the total amount of released DOX was maximum when the monomer ratio was 5 mol % and crosslinker ratio was 1 mol % in the AA-TEGDMA based hydrogels. On the contrary, when 1 mol % AA and 5 mol % TEGDMA were

used, the amount of released DOX had a minimum value. The employment of 5 mol % crosslinker had no practically effect on the release amount with increasing monomer ratio (Figure 5.52).

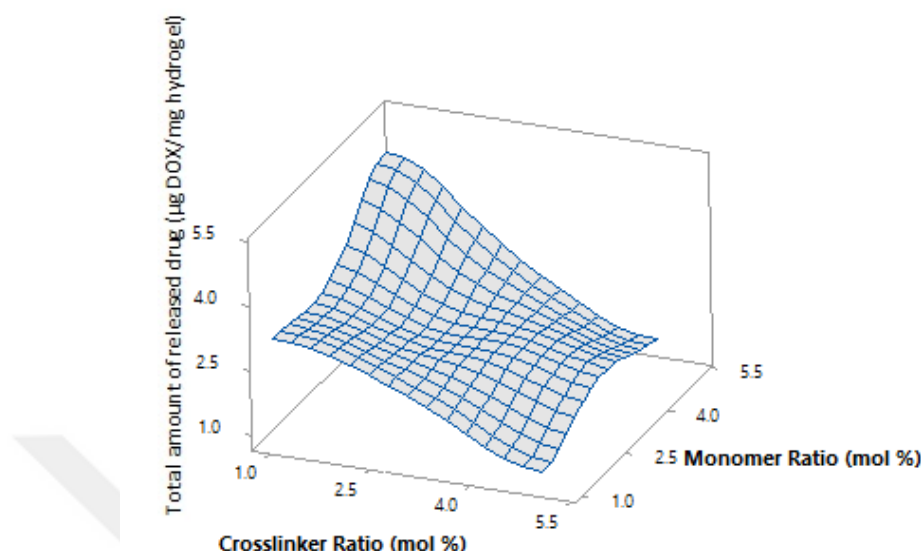


Figure 5.52 : 3D surface plot for crosslinker- monomer interaction in AA-TEGDMA based hydrogels.

Increasing the monomer ratio for non-imprinted hydrogels slightly increased amount of the released DOX. When the monomer ratio was 5 mol %, more drug released for the non-imprinted hydrogels. On the other hand, increasing the AA:DOX ratio led to an increase in the releasing amount at a constant monomer ratio for the imprinted hydrogels. This effect was even more pronounced as the monomer ratio increased to 5 mol % and AA:DOX ratio was equal to 20. This case showed that not only the M:T ratio but also the amount of monomer contributed to the quantity of released drug with respect to non-imprinted hydrogels. When AA:DOX ratio was 31.25, there was a significant decrease in the amount of released DOX between imprinted and non-imprinted hydrogels. Moreover, the amount of DOX release from imprinted hydrogel was lower than non-imprinted counterpart. Similar to AA-EGDMA hydrogels 5 mol % functional monomer, 5 mol % crosslinker containing imprinted hydrogel released less drug than non-imprinted counterpart. (Figure 5.53). Randomly distributed functional monomers reduced the specificity of imprinting and destroyed well-organized structure of the hydrogel. Also, it may be related with immobilization of the drug molecules because of excess monomer ratio.

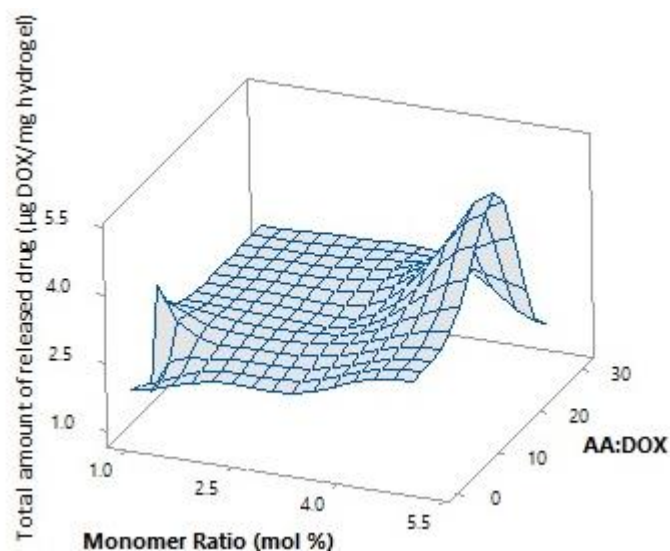


Figure 5.53 : 3D surface plot for monomer ratio AA:DOX ratio interaction in AA-TEGDMA hydrogels.

On the other hand, cumulative released DOX decreased for both imprinted and non-imprinted hydrogels when the crosslinker ratio was increased from 1 to 5 mol %. The imprinted hydrogels released greater amount of drug than non-imprinted hydrogels at a constant crosslinker ratio until AA:DOX was equal to 20 . The amount of released DOX decreased for each crosslinker ratio since AA:DOX was 31.25. Reduction of released quantity was noteworthy often when the crosslinker was 5 mol % (Figure 5.54).

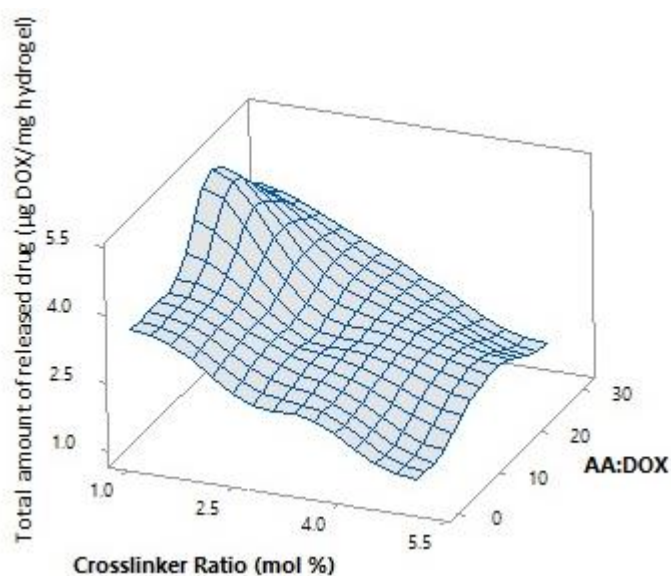


Figure 5.54 : 3D surface plot for crosslinker ratio AA:DOX ratio interaction in AA-TEGDMA hydrogels.

The well-defined coordination of the functional monomer units in the cavities allowed the binding affinity between template and functional monomer to increase with

increasing the number of binding sites. Well-shaped cavities were utilized via molecular imprinting and reduced interaction of DOX with crosslinker. Therefore, the strong interactions between AA and DOX decreased the number of DOX-TEGDMA interactions in the hydrogel network. Fewer cavities but stronger affinity may be created via TEGDMA crosslinker. It was estimated that the number of randomly distributed AA monomers in the matrix were less for imprinted hydrogels for AA-TEGDMA based hydrogels. This means that a large number of AA monomers were incorporated into the cavities specifically. TEGDMA with AA may allow the drug to fix better in the cavities and tighter structure caused to a slowly drug release with respect to AA-EGDMA based hydrogels.

In the literature, it was suggested that if the crosslinker had lower affinity towards the template molecules, it would be expected that the molecular imprinted polymers will show lower non-specific interaction with template and enhance higher imprinting efficiency [216,217]. Our obtained results showed that TEGDMA-based imprinted hydrogels gave better results even though they had relatively stronger interaction with DOX than EGDMA for certain X:T ratios (for low crosslinker concentrations) during UV-Vis studies. It was observed that not only interaction with drug molecules but also structural arrangements significantly were effective on drug release performance of the hydrogels.

Release curves of IA based hydrogels with EGDMA (hydrogel 6-10) and TEGDMA (hydrogel 16-20) were plotted as a function of time and presented at Figure 5.49 (b-d). As expected, IA containing imprinted and non-imprinted hydrogels showed higher cumulative drug release than AA containing imprinted and non-imprinted counterparts (Table 5.33). Contrary to the loading results, IA-TEGDMA based hydrogels (Hydrogel 16-20) had the ability to release more drug than IA-EGDMA (Hydrogel 6-10) based hydrogels. One of the possible explanations was that the IA-TEGDMA containing hydrogels absorbed more water and led to release more drug than the IA-EGDMA based hydrogels. The volume fraction of swollen state in IA-TEGDMA based hydrogels decreased more than IA-EGDMA based ones which means that more water entered into the structure. Furthermore, it indicated that there was sufficient open transport path for diffusion of the drug.

IA-EGDMA based hydrogels released greater amount of DOX when functional monomer ratio was high and crosslinker ratio was low for both NIPs and MIPs (Figure

5.55). High crosslinker ratio prevented water absorption and lowered amount of drug was released.

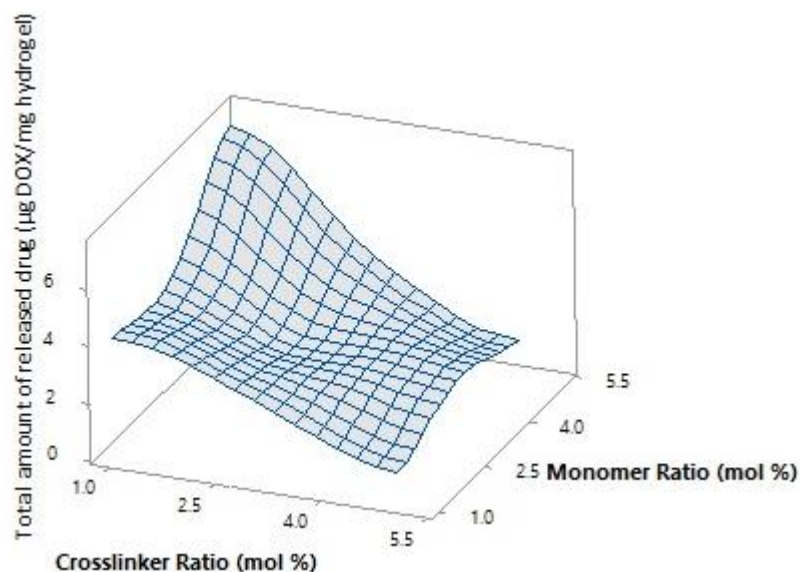


Figure 5.55 : 3D surface plot for crosslinker- monomer interaction in IA-EGDMA hydrogels.

The non-imprinted IA-EGDMA containing hydrogels released more amount of drug when monomer ratio increased from 1 mol % to 5 mol % by mol (Figure 5.56). The differences between the imprinted and non-imprinted hydrogels became maximum when M:T ratio was equal to 14.6 (MIP released 55.4 % greater DOX than NIP). This fact confirms that the M:T ratio governed the cumulative drug release. Additionally, not only M:T ratio but also monomer ratio was important for succesful preparation of IA-EGDMA based MIPs. At low monomer ratio with equal M:T ratio imprinted and non-imprinted hydrogels released nearly equal amount of DOX. For the low monomer ratio, the impact of imprinting on the delivery of DOX was not significant. Above 14.6 for M:T ratio with high monomer ratio (M:T is equal to 20 and 31.25), amount of released drug from imprinted hydrogel decreased drastically. This case was attributed to non-specific interactions due to a large excess functional monomer which unveil EGDMA-IA and IA-IA interactions. The lack of the monomer moiety in the cavities caused to insufficient imprinting efficiency. These two cases caused that synthesis of the hyrogel in the presence of the drug did not modify the releasing behavior.

It was likely that the excess monomer increased the number of EGDMA-IA or IA-IA reactions during the polymerization in the MIP. This would reduce the number of specific DOX-IA interactions, which did not allow to form sufficient binding sites in the polymer. Having a slight IA excess in polymerization mixture during the

polymerization could optimize the number of interactions between the template and IA functional groups, resulting in high number of binding sites.

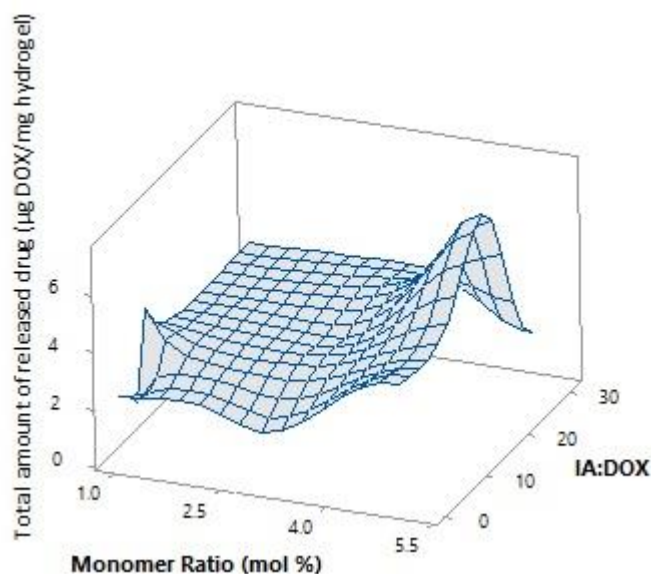


Figure 5.56 : 3D surface plot for monomer ratio AA:DOX ratio interaction in IA-EGDMA hydrogels.

When the crosslinker proportion was low, the effect of imprinting on the release of DOX was clearly obvious. Maximum releasing occurred when EGDMA was 1 mol % and M:T ratio was 20. However, imprinted hydrogel released more drug than non-imprinted counterparts when M:T was 14.6. At a fixed M:T ratio, increasing the crosslinker ratio resulted in a reduction of released drug. (Figure 5.57). For all crosslinker ratios, released amount of drug increased with increasing M:T until 14.6. After this point, drug release performance remained nearly constant or reduced according to the crosslinker ratio. The excess of crosslinker caused to more rigid structure and non-covalent EGDMA-IA interaction as well as interaction between AA-DOX prevented via crosslinker. Limiting ionization also caused a decline in cumulative released DOX.

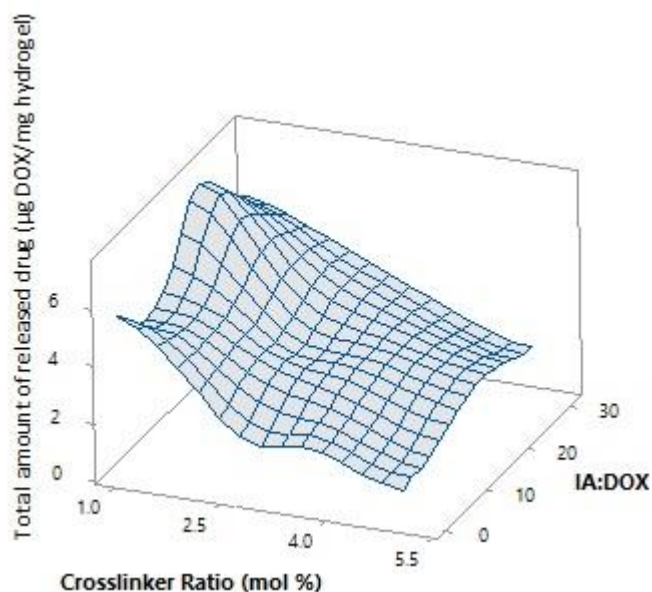


Figure 5.57 : 3D surface plot for crosslinker ratio IA:DOX ratio interaction in IA-EGDMA hydrogels.

It is noteworthy to state that the loading results of the hydrogels synthesized with IA and TEGDMA had quite similar trend with IA-EGDMA based hydrogels. As the IF value approached to 1, the non-imprinted hydrogel released more drug than the imprinted one (hydrogel 17 and 18) (Table 5.33). Large number of IA arranged in the specific cavities and further TEGDMA helped to effective enclosure of the template in the cavity. In a study by Gryshchenko and coworker, the lowest porosity was measured for TEGDMA imprinted hydrogels. This case indicated that TEGDMA based imprinted hydrogels had a higher mass concentration in the pre-polymerization mixture and led to become a more dense polymer.

The non-imprinted IA-TEGDMA based hydrogels released more amount of drug when monomer ratio was high with low crosslinker ratio (Figure 5.58). Additionally, high amount of crosslinker caused to prevent ionization and was to be decreased water absorption and releasing drug although it had high amount of monomer.

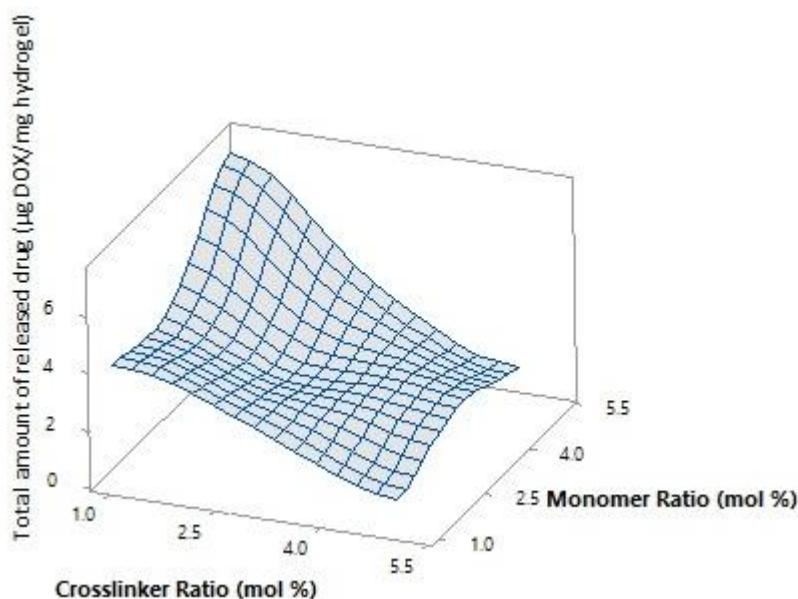


Figure 5.58 : 3D surface plot for crosslinker- monomer interaction in IA-TEGDMA hydrogels.

It can be seen from Figure 5.59, the imprinted hydrogels released more amount of DOX than non-imprinted ones when monomer was used higher than 1 mol %. If the IA:DOX ratio was taken as 31.25 and the amount of monomer was increased, cumulative amount of drug released was increased notably.

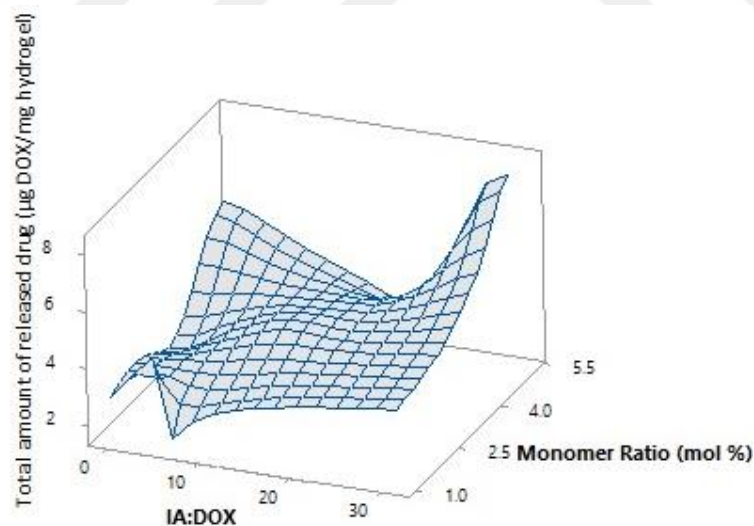


Figure 5.59 : 3D surface plot for monomer ratio IA:DOX ratio interaction in IA-TEGDMA hydrogels.

The effectiveness of the cavities were also affected by the template:monomer ratio. Above 14.6 as M:T ratio (M:T was equal 20 and 31.25) with high monomer ratio (5 mol %) the difference between imprinted and non-imprinted hydrogel was become pronounced. This occurrence can be attributed to the saturation of the cavities with monomers when M:T was equal to 20 or 31.25. IA-DOX interaction became more

available and non-specific interactions were minimized. However, hydrogel synthesis in the presence of the drug did not modify the loading capability of IA-TEGDMA based hydrogels. Imprinted hydrogels network with less non-specific interaction led the cumulative released DOX to rise with respect to non-imprinted counterparts. It may be said that non-specific irreversible interactions (IA-TEGDMA, DOX-TEGDMA and IA-IA interactions) were eliminated.

IA-TEGDMA based hydrogels absorbed more water due to ionization of -COOH groups in the releasing medium, $V_{2,s}$ decreased noticeable and more drugs were released from the free diffusion paths. As a result, drug, monomer and crosslinker interaction had an active role in structural organization.

As observed in previous results, increasing the crosslinker ratio caused to a decrease in the amount of released drug in 48 h (Figure 5.60). When IA:DOX ratio was kept at a certain value, it was observed that every time low crosslinked hydrogel released more drug than highly crosslinked hydrogel. On the other hand, hydrogel with IA:DOX equal to 31.25, increased released DOX significantly at low crosslinked hydrogel with respect to the non-imprinted one.

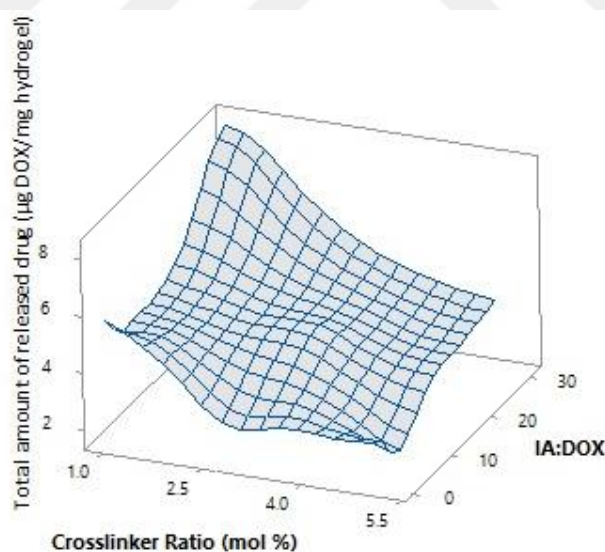


Figure 5.60 : 3D surface plot for crosslinker ratio AA:DOX ratio interaction in IA-TEGDMA hydrogels.

5.3.4.2 Mathematical modelling of drug release results

In the study, the drug released quite fast during approximately 120-180 minutes which led to the rapid increase of drug concentration in the solution. This demonstrated that the drug experienced fast release at first, thereafter moved into a sustained release period and consequently a final strengthened release in the third period. This last phase

was negligible due to very slow rate. Biphasic drug delivery system was composed of one fast release period and one sustain release period. In this case, the first release period was slowed down and the second period was approximated via molecular imprinting technique. Cumulative percentage of DOX releasing from all hydrogels were presented in Figure 5.61. Generally, 45-80 % drug released in 6 hours for all hydrogels depending on the composition and imprinting.

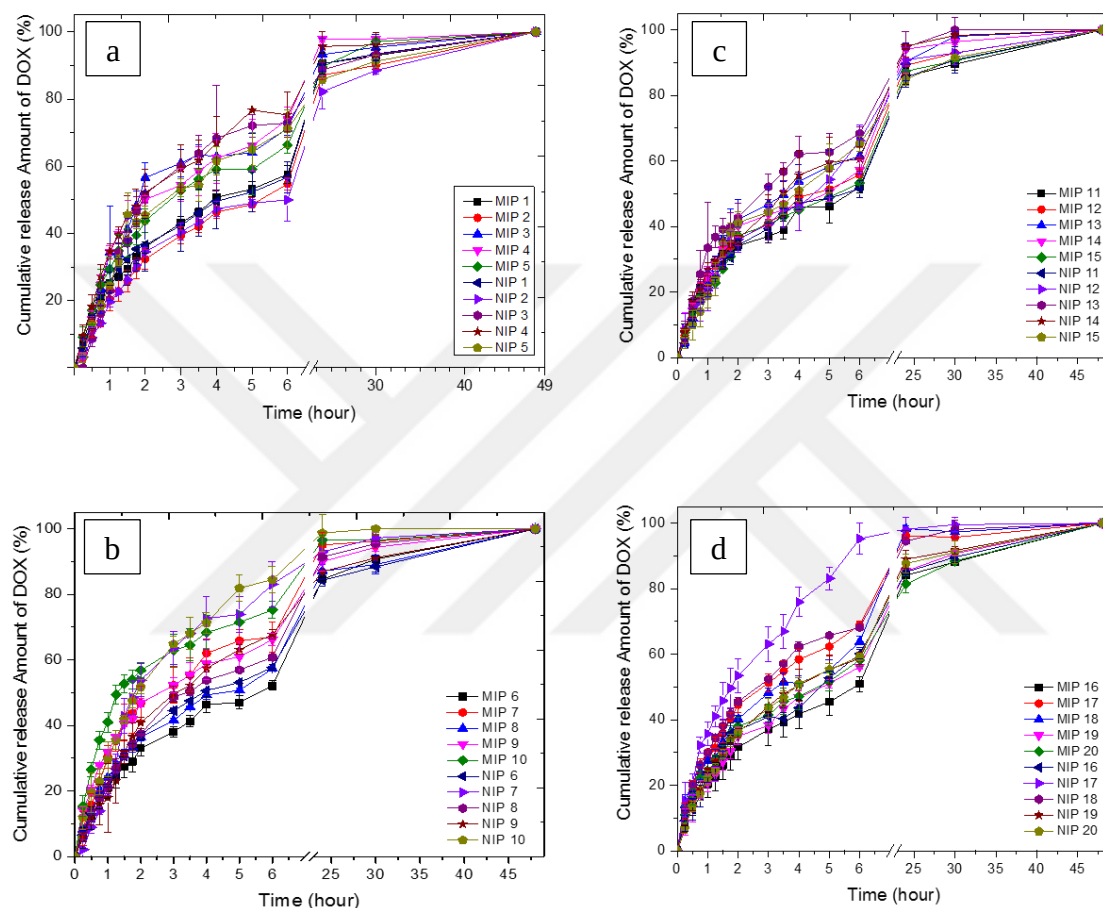


Figure 5.61 : Cumulative releasing profiles of the hydrogels for a:AA-EGDMA b:IA-EGDMA c:AA-TEGDMA and d: IA-TEGDMA containing hydrogels both imprinted and non-imprinted.

In our study, a model-dependent approach was used to clarify drug release mechanisms and kinetics. To apply the model-dependent approach, drug release data were fitted to two popular models: Higuchi and Korsmeyer Peppas (Table 5.33 and 5.34). The goodness of fit in different release kinetic models (Korsmeyer, zero-order and Higuchi models) was evaluated to understand the mechanism of drug release (Figure 5.37 and 5.24). When the fraction of DOX released was plotted against time, according to

Equation (4.20), the plots did not present satisfactory linearity (Figure 5.62 a-d); the releasing mechanism was not similar to zero order transport.

Table 5.1 : Regression coefficients and n values obtained from Higuchi plot.

	n	R²		n	R²
NIP 1	24.81	0.975	MIP 1	24.89	0.929
NIP 2	24.37	0.962	MIP 2	24.03	0.947
NIP 3	37.16	0.938	MIP 3	32.72	0.977
NIP 4	34.03	0.973	MIP 4	32.40	0.984
NIP 5	32.56	0.951	MIP 5	28.71	0.969
NIP 6	25.89	0.982	MIP 6	22.90	0.987
NIP 7	40.50	0.940	MIP 7	30.36	0.977
NIP 8	27.72	0.983	MIP 8	24.36	0.938
NIP 9	31.42	0.972	MIP 9	27.49	0.970
NIP 10	38.23	0.986	MIP 10	31.04	0.981
NIP 11	23.60	0.976	MIP 11	21.12	0.965
NIP 12	26.32	0.979	MIP 12	24.01	0.967
NIP 13	29.74	0.976	MIP 13	28.33	0.982
NIP 14	27.04	0.988	MIP 14	24.00	0.997
NIP 15	28.90	0.960	MIP 15	23.14	0.992
NIP 16	24.34	0.972	MIP 16	20.87	0.990
NIP 17	38.91	0.994	MIP 17	29.01	0.993
NIP 18	29.64	0.989	MIP 18	25.71	0.989
NIP 19	26.08	0.989	MIP 19	23.96	0.929
NIP 20	24.81	0.989	MIP 20	23.71	0.947

Table 5.38 showed the correlation of drug release datas to Higuchi models. The lowest value of R^2 was 0.929. The high values of the coefficient of linear regression for the Korsmeyer model confirmed (Table 5.39) that the data treatment could be used validly for hydrogel films. The regression coefficients for the Korsmeyer Peppas model were generally higher than those for the Higuchi model for hydrogels. The drug release mechanism for imprinted and non-imprinted systems has been explained by using the Korsmeyer equation based on the best regression coefficient values (R^2). The values of the release exponent (n), kinetic rate constant (k) and the regression coefficient (R^2) were gathered in the Table 5.35. The lowest value of regression coefficient of Korsmeyer-Peppas model was 0.9428. Diffusion and polymer relaxation was covered by this model successfully. When n value is equal to 0.45, Fickian diffusion occurred by the usual molecular diffusion of the drug due to a chemical potential gradient [180]. Swelling (Zero order) controlled drug release occurred when value of $n=0.89$. That was

the release of the drug associated with the expansion of the state-transition in hydrophilic glassy polymers which swell in water or biological fluids . In the case of $0.45 < n < 0.89$, non-Fickian diffusion occurred which was the combination of both drug diffusion and polymer relaxation in order to administer drug release rate. Diffusion rate of the drug molecules from the polymers and relaxation time of the polymer chains were both comparable. If the n value was bigger than 0.89, it mean that drug release was controlled via Super case II transport mechanism. In the Super case II transport mechanism diffusion and relaxation occurred but relaxation of the polymer chains were dominant for controlling mechanism.

Plot of $\ln(M_t/M_\infty)$ against $\ln(t)$ were shown in Figure 5.62 for the MIP 19 and NIP 19 as an example. As it can be seen, there were two linear releasing pattern. The first phase relatively had large slope value. The slope of first lines were 0.9307 and 0.6313 for NIP 19 and MIP 19, respectively, representing the diffusion exponent, n , which showed that the DOX released from MIP with non-Fickian diffusion and from NIP with super case II transport mechanism. Diffusion coefficient of imprinted network was lower than the non-imprinted network. These results were given in Figure 5.47. The controlling of the drug release from imprinted hydrogels can be determined by the diffusion process of drug partially through the molecular imprinted cavities in the polymer network, as well as the relaxation of the HEMA based hydrogel network.

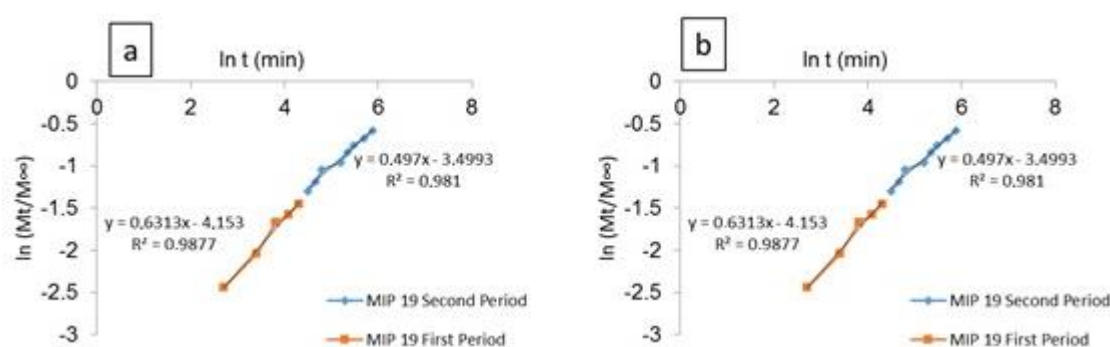


Figure 5.62 : Slope of Korsmeyer Peppas for a: MIP 19 b: NIP19.

It can be clearly seen that, the values of ' n ' were greater than 0.45 in each release case for both imprinted and non-imprinted hydrogels. In this case, the release process was significantly driven by both diffusion and swelling of the DOX molecules (Table 5.35).

Figure 5.30 demonstrateds the pH sensitivity of the hydrogels. These results showed that swelling behavior of pH sensitive hydrogels changed with medium acidity. Both IA and AA had ionaziabale groups with different pKa values. The swelling studies showed that swelling

ratio was higher noticeably at pH=7.0 (releasing medium) than at pH=3.9 (loading medium). Relaxation of the polymer network was affected by the ionization degree of the polymer networks. For our study the ionization was noticeable and relaxation became a dominant controlling mechanism for DOX releasing especially for non-imprinted hydrogels (Table 5.35). In the literature similar results were obtained by other research groups. Meth acrylic based pH sensitive hydrogels were investigated by Bartil et al, to define the mechanism of water transport through hydrogel matrix [246]. Their results showed that pH affects water transport mechanism significantly. At pH 7.0, Super case II controls the transport mechanism whereas at pH 1.2, the non-Fickian mechanism was dominant. A similar study has been performed by Kim et al. and the matching result has been obtained for pH-sensitive anionic hydrogels used for protein delivery [247].

Our statistical analysis results indicated that imprinting effect led to a decrease of n value below 0.89 as it was mentioned in Section 5.3.1.4. Furthermore, TEGDMA based hydrogels had lower n value than EGDMA based hydrogels. IA containing both non-imprinted and imprinted hydrogels reduced n value. It was also indicated that monomer type played an important role on altering drug transport mechanism (Table 5.35). These results showed that releasing DOX can be tailored via the binding sites within the hydrogel matrix due to imprinting process. The DOX transport mechanism was controlled by nature, ratio and arrangement of functional monomer with nature of crosslinker and its ratio.

Using imprinting technique mostly changed releasing mechanism from super case II transport to non-Fickian transport. The arrangement of the monomers in the imprinted cavities led to creation of high affinity to DOX (Figure 5.48). NIP presented higher diffusion coefficient due to low monomer-drug interactions and structural differences. This suggested that not only affinity between template and functional monomer were necessary for fabrication of imprinted polymer-drug complexation but also diffusion path and structural alteration had a significant role on controlling drug release mechanism. Imprinted hydrogels had more ordered functional groups in the cavities. n values of non-imprinted hydrogels were greater than molecular imprinted hydrogels except for hydrogel 4, 10 and 12. However, both imprinted and non-imprinted hydrogels released drug by the same transport mechanism (super case II) for Hydrogel 4 and 10. The transport mechanism for imprinted and non-imprinted hydrogel changed for only hydrogel 12. Although NIP and MIP of hydrogel 12 had similar diffusion

coefficients (Figure 5.47), imprinted hydrogel 12 had lower $V_{2,s}$ value than non-imprinted counterpart.



Table 5.2 : n and k values obtained from Korsmeyer Peppas Equation for imprinted and non-imprinted hydrogels.

Hydrogel Code	NIPs						Hydrogel Code	MIPs					
	n ₁	k ₁	R ²	n ₂	k ₂	R ²		n ₁	k ₁	R ²	n ₂	k ₂	R ²
1	1.0806	0.0032	0.9946	0.4097	0.0512	0.9945	1	0.8238	0.0088	0.9966	0.4862	0.0340	0.9903
2	1.1877	0.0013	0.9847	0.4033	0.0492	0.0955	2	0.975	0.0039	0.9919	0.5387	0.0234	0.9871
3	1.3864	0.0007	0.9907	0.4353	0.0626	0.9800	3	1.2201	0.0022	0.9997	0.6176	0.0261	0.8956
4	0.9094	0.0081	0.9962	0.3579	0.0918	0.9880	4	0.9765	0.0004	0.9838	0.3473	0.0920	0.9737
5	1.4252	0.0007	0.9681	0.3990	0.0672	0.09842	5	0.8328	0.0098	0.9951	0.4119	0.0596	0.9687
6	0.9346	0.0072	0.9867	0.4447	0.0431	0.9852	6	0.8756	0.0040	0.9914	0.4752	0.0326	0.9857
7	1.3703	0.0003	0.9900	0.3777	0.0892	0.9756	7	0.8954	0.0075	0.9989	0.3877	0.0717	0.9838
8	0.759	0.0099	0.9976	0.3249	0.0897	0.9909	8	0.7493	0.0109	0.9954	0.4220	0.0473	0.9851
9	0.9107	0.0049	0.9828	0.4648	0.0442	0.9905	9	0.6039	0.0270	0.9957	0.3546	0.0827	0.9799
10	0.7026	0.0172	0.9937	0.7026	0.017	0.9937	10	0.7192	0.0223	0.9940	0.2727	0.1530	0.9932
11	1.1539	0.0020	0.9814	0.4677	0.0346	0.9736	11	0.7981	0.0096	0.9809	0.3654	0.058	0.9518
12	0.6873	0.0134	0.9734	0.6704	0.012	0.954	12	0.8889	0.0064	0.9851	0.3980	0.0533	0.9908
13	1.0176	0.005	0.9991	0.4301	0.055	0.9789	13	0.9136	0.0023	0.9851	0.3835	0.0648	0.9909
14	0.894	0.0072	0.9706	0.5007	0.0337	0.9863	14	0.7627	0.010	0.9986	0.3815	0.0588	0.9428
15	0.89	0.0049	0.9963	0.3740	0.0658	0.9643	15	0.685	1.2671	0.9882	0.3289	7.5104	0.9768
16	0.7152	0.0121	0.9793	0.6075	0.0164	0.9271	16	0.519	0.0247	0.9953	0.4365	0.0268	0.9951
17	0.738	0.0262	0.9481	0.4873	0.0499	0.9915	17	0.6018	0.0241	0.9888	0.4146	0.0596	0.9922
18	0.7353	0.0226	0.9950	0.4703	0.0466	0.99000	18	0.4991	0.0352	0.9872	0.4696	0.0401	0.9702
19	0.9307	0.0055	0.9967	0.4978	0.0326	0.9921	19	0.6313	0.0157	0.9877	0.4970	0.0302	0.9810
20	0.7898	0.0115	0.9905	0.4873	29.023	0.9927	20	0.5905	0.0221	0.9918	0.4643	0.0373	0.9830

When loading-releasing behaviors and n values were evaluated together, the results were significantly satisfactory. Loading performances directly affected the quantity of released DOX and n value. It contained the lowest IF value among AA-TEGDMA based hydrogels. The swelling may caused an increase in mobility of drug molecules. It can be said that molecular imprinted hydrogel 12 had enough long diffusion path but lack binding sites which led to a acceleration in drug release.

It was obviously clear that the effects of TEGDMA and EGDMA as crosslinker were different on the releasing mechanism of the hydrogels. This proposed that for EGDMA based non-imprinted hydrogels the mechanism of the release was dominantly super case II transport, for TEGDMA based non-imprinted hydrogels the mechanism was dominantly non-Fickian diffusion transport (Table 5.35). It was demonstrated that the mesh size of the TEGDMA based hydrogels were smaller than EGDMA based hydrogels. Monomers became relatively closer to drugs with using TEGDMA. Shortening of the distance between the monomer and template molecule allowed to slow diffusion by moving slowly drug molecules from one memory site to another. On the other hand, using TEGDMA can eliminate the non-specific interactions between crosslinker and drug molecules.

The mechanism of the release was super case II transport for AA-EGDMA based non-imprinted hydrogels. Imprinting changed the transport mechanism to non-Fickian only for hydrogel 1 and hydrogel 5. At high crosslinker and low monomer ratios, NIPs and MIPs exhibited similar releasing pattern. (Figure 5.63 a and b). The lowest n value ($n=0.8328$) obtained for hydrogel 5 which had highest imprinting factor and the highest difference in releasing amount between NIP and MIP. The imprinted matrix had lower diffusion coefficient than the non-imprinted one for hydrogel 5, in spite of comparable mesh size and polymer volume fraction in the swollen state. It can be strongly said that imprinting affected the arrangement of functional groups interacting with the template for AA-EGDMA based hydrogels. Additionally, the optimum conditions helped to reduce non-specific irreversible interactions which caused to increase DOX-AA interactions. The AA-EGDMA based imprinted hydrogels increased the released amount of DOX than non-imprinted counterparts (except hydrogel 3 and 4). However, these samples remained weak in the control of drug

release. It was attributed to the fact that AA did not interact enough with DOX molecules through diffusion path.

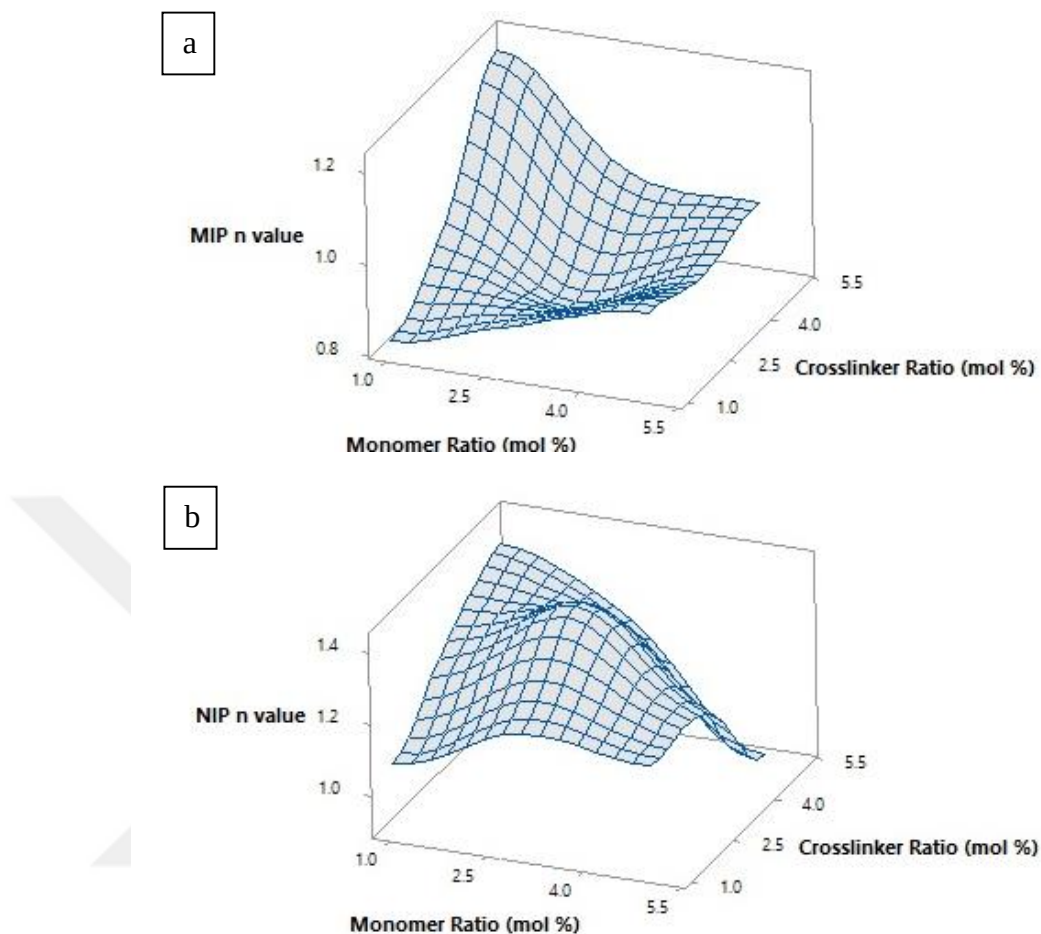


Figure 5.63 : 3D surface plots for n values in AA-EGDMA hydrogels a. imprinted b. non-imprinted for M:X effect.

n values of AA-TEGDMA based hydrogels were lower than AA-EGDMA based hydrogels. It was clear that the effects of TEGDMA and EGDMA as crosslinkers were different on the imprinting process. Increasing the number of arranged monomer in the cavity attributed to shortening of the distance between the monomer and template molecule. Randomly distributed AA decreased with respect to the AA-EGDMA including hydrogels. However, higher affinity binding sites were created and led to more controllable drug release. On the other hand, swelling ratio for AA-EGDMA hydrogels was higher than AA-TEGDMA hydrogels: It means that drug release was more controlled.

AA-TEGDMA based MIPs had a distinctly different release behavior from the NIPs counterparts when and crosslinker ratio changed (Figure 5.64). When AA and TEGDMA was used to synthesize hydrogels (hydrogel 11-15), all MIPs released drug

with non-Fickian transport except for hydrogel 13. MIP 13 had higher diffusion coefficient with respect to the other AA-TEGDMA hydrogels and interestingly loaded higher amount of DOX than non-imprinted counterpart. And also, NIPs released drug with super case II transport except for hydrogel 12. Hydrogel 12, had the lowest imprinting factor and highest releasing amount as imprinted hydrogel, meanwhile it had the highest n value.

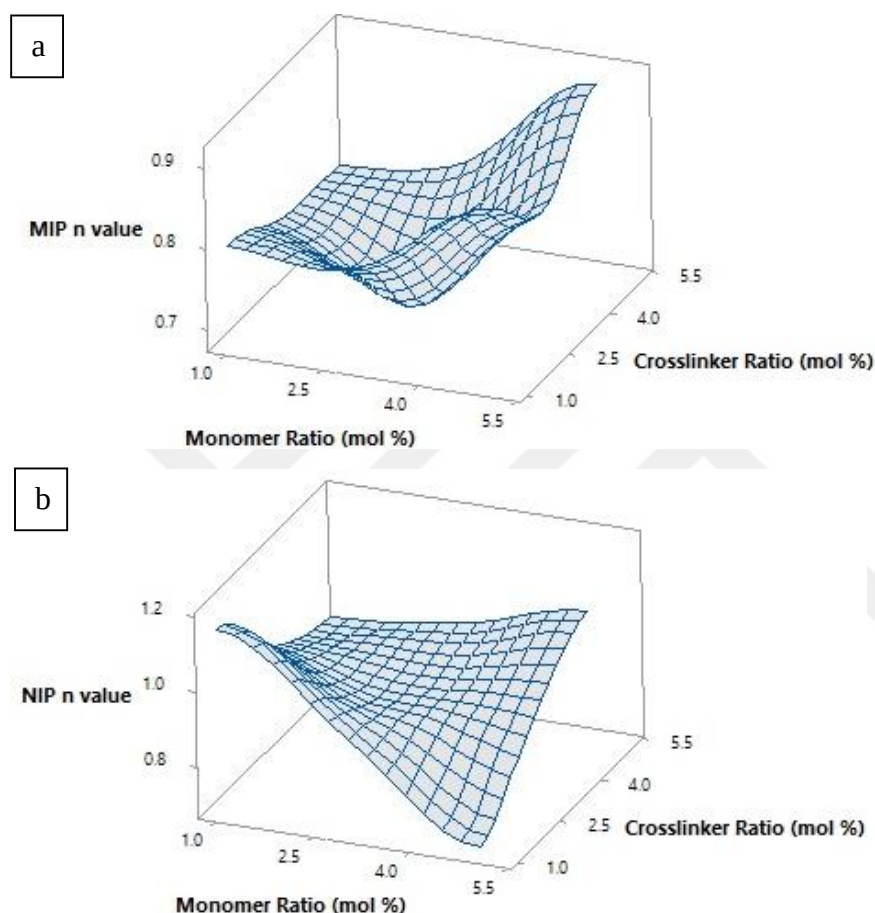


Figure 5.64 : 3D surface plots for n values in AA-TEGDMA hydrogels a. imprinted b. non-imprinted for M:X effect.

It was seen that only hydrogel 13 exhibited similar releasing pattern when NIP and MIP 13 was compared. This hydrogel had 5 mol % TEGDMA and 5 mol % AA in the hydrogel preparation step. Diffusion coefficient of hydrogel 13 was the highest which indicated that high crosslinker and monomer ratio caused to fast release. Limiting swelling ratio and diffusion path length with rigid structure caused to uncontrollable drug release. The lowest n value ($n=0.68$) obtained for imprinted hydrogel 15 which non-imprinted counterpart had lowest n value.

Diffusion coefficients were lower than the AA-EGDMA based hydrogels and, also polymer volume fraction was higher than AA-EGDMA based hydrogels. This indicated that AA-TEGDMA based hydrogels had tighter diffusion path than AA-EGDMA counterpart and this case delayed drug transport.

The effect of high DOX-IA interaction can be clearly understood in the releasing performances. All IA-EGDMA (hydrogel 6-10) based imprinted hydrogels (except hydrogel 7) released DOX by non-Fickian transport. In the case of using IA as functional monomer, number of IA in the cavities and affinity of binding sites against DOX may led to required DOX binding. IA made stronger interaction with DOX than AA molecules as a consequence of large number of functional groups and greater affinity. It appeared that more number of IA tend to interact with one DOX molecule. Therefore, excess number of monomers was required due to saturation of the cavities. When low monomer ratio with high crosslinker ratio was employed, DOX release were dominantly carried out by swelling controlled mechanism (hydrogel 7) (Figure 5.66). At a constant low crosslinker ratio, n slightly increased with decreasing monomer ratio. The obtained result suggested that interaction of itaconic acid and DOX can be expected without applying imprinting. The imprinting enhanced controlled release of DOX by using IA together.

The highest n value ($n_{MIP}=0.8954$ $n_{NIP}= 1.134$) indicated Super case II transport mechanism for hydrogel 7. Hydrogel 7 had lowest imprinting factor and highest difference in releasing amount as percentage between NIP and MIP (Table 5.33). As it can be seen from Figure 5.47, both imprinted and non-imprinted hydrogel 7 networks had highest diffusion coefficients and volume fraction in swollen state as well as the smallest mesh size with respect to the other IA-EGDMA based hydrogels. The result for hydrogel 7 demonstrated that the imprinting enhanced the controlling of the drug transport. But the inadequate number of monomers in the cavities ($M:X=4$) reduced the effect of imprinting on the releasing kinetic. It appeared that imprinting affected the arrangement of functional groups interacting with the template for IA-EGDMA based hydrogels.

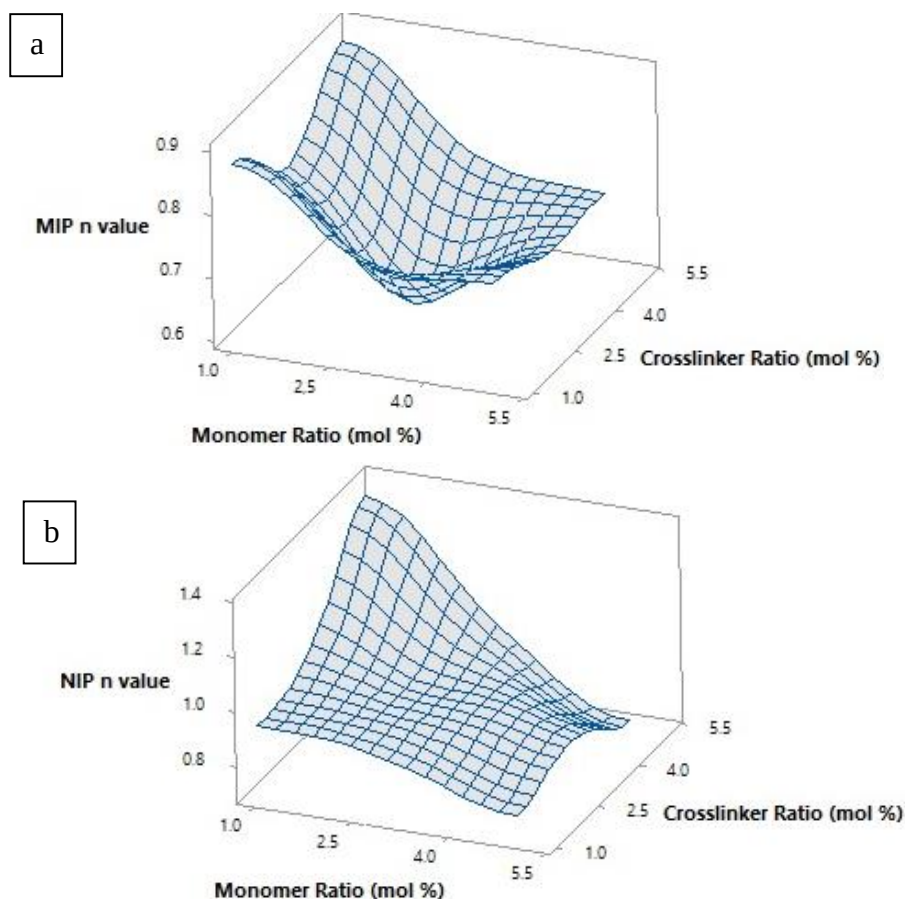


Figure 5.65 : 3D surface plots for n values in IA-EGDMA hydrogels a. imprinted b. non-imprinted for M:X effect.

IA-TEGDMA including both MIP and NIPs had lower n values than other crosslinker-monomer combinations (Table 5.35). This case indicated that both MIPs and NIPs released drug by non-fickian diffusion transport mechanism (except non-imprinted hydrogel 19). All non-imprinted hydrogels had greater diffusion coefficient than imprinted counterparts. As it can be seen from Figure 5.67, increasing the amount of functional monomer at low crosslink ratio resulted in decreasing the volume fraction in swollen state which the drug diffused fastly without distraction in cavities for non-imprinted hydrogel 19. Also, M:T ratio imprinted hydrogel was equal to 31.25 and result in lowering mesh size and diffusion coefficient as a consequence of preparing high affinity binding sites. The multiple functional monomer-DOX interactions were believed to enhanced affinity and lower diffusion coefficients. On the other hand, low crosslinker ratio led to decrease the interaction strength (hydrogen bond or electrostatic) due to increase distance between monomer and drug molecule.

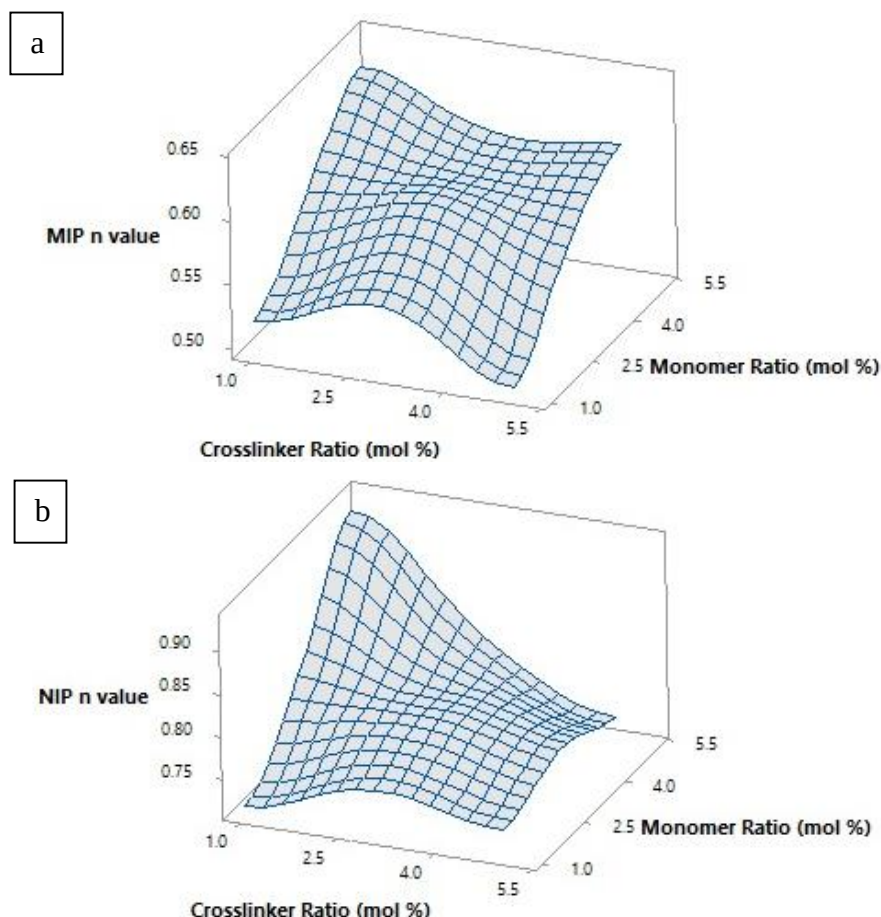


Figure 5.66 : 3D surface plots for n values in IA-TEGDMA hydrogels a. imprinted b. non-imprinted for M:X effect.

As shown in Figures 5.49 (a-d), the hydrogel continued to release DOX for 48 hours. The non-imprinted hydrogels released drug fastly in the first phase. The imprinted hydrogels released DOX more controllable than NIPs, in general. This pattern demonstrated the advantage of applying molecularly imprinting approach into hydrogels as delivery systems. The release pattern and its period can be programmed by the imprinting technique. Thus, it was possible to release at similar or different rates in both phases depending on the hydrogel composition [189].

When the n value was higher at the first releasing phase, it caused a decrease in n value below 0.45 at the second releasing phase which indicated to Fickian transport. The slopes of the first linear portion of the Korsmeyer Peppas plots pointed that about 40 % of the drug was released within 1-3 hours depending on hydrogel composition. It was expected to achieve extend release of DOX thereafter. The molecular imprinting process had made considerable contribution in the first step to control drug release. Moreover, it was required to continue the drug release in a controlled method in the second releasing phase.

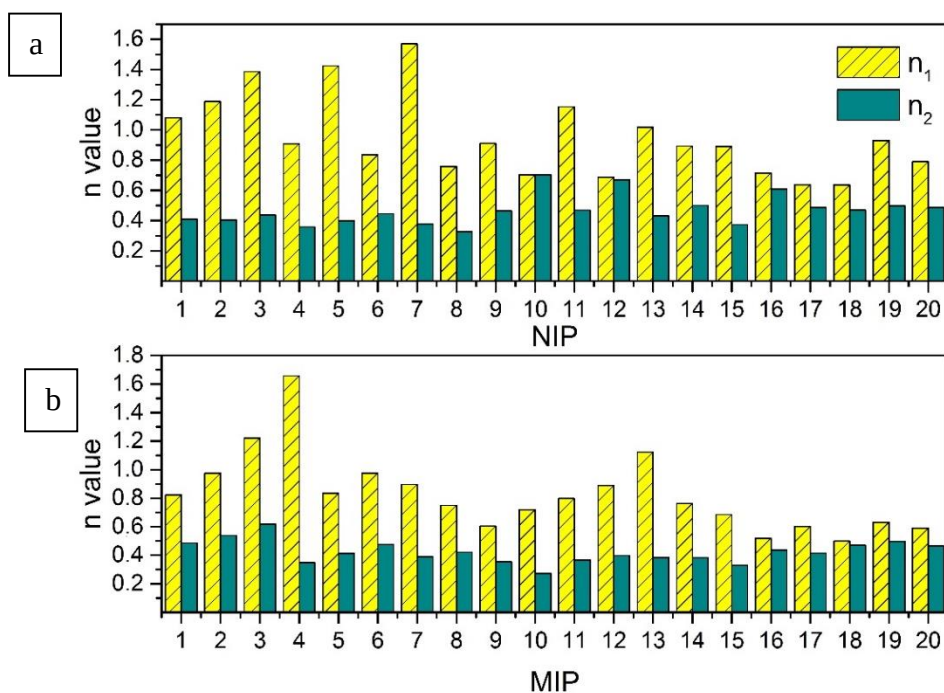


Figure 5.67 : n values hydrogels for first and second releasing steps.

Figure 5.67 represented that n values for first and second releasing phase for both NIPs and MIPs. However for the TEGDMA crosslinked matrices the n values of hydrogels were lower, drug released more controlled, compared to EGDMA including hydrogels. Additionally, it can be seen clearly from Figure 5.68, n values of TEGDMA-IA based hydrogels are lower than the other hydrogels. Also first and second phase n values became close to each other by imprinting.

We observed a wide range of release among different hydrogel based on the released DOX results. The cumulative data sets included more data points and analysed both mathematical and statistical. Having such a kind of highly wide range experimental set helped to analysis interactions and differences between the variables. The mathematical modelling analysis gave information about drug release transport mechanism. And it was seen transport mechanism changed effectively with changing selected experimental variables. Both cumulative release, mathematical modelling and statistical analysis results indicated that IA and TEGDMA were the optimal monomer and crosslinker combination in order to imprint successfully DOX molecule for controlled drug release.

Indeed, it was an exciting task to prepare effective MIPs for DOX since it stand as a large molecule with many special features in terms of shape and functionality. It was also a kind of charged molecule with three Pka values. On the other hand, it had

capability to make intramolecular hydrogen bonding because of its conformation (Figure 3.5). The targeted imprinting process which was accomplished successfully provided that the selected monomer and crosslinker were performed appropriately. This was the product of the effective combination of the imprinting method together with the employment of the favorable functional monomer and crosslinker to bind specifically the drug to the polymeric matrix. All these factors made the pre-polymerization complex more stable, which resulted in more selective binding sites in final MIP network, and, consequently, a stronger imprinting effect and controlled drug release was observed. However, the strength of template-monomer complex was affected by the number of monomers, crosslinkers and template molecule presence in polymerization mixture and the affinity of the functional groups on the monomers after polymerization.

In the next part, we studied with IA and TEGDMA as functional monomer and crosslinker with two constant crosslinker ratio as 1 mol % and 5 mol%. This monomer-crosslinker combinations was selected according to experimentally and statistically obtained results. These studies were important to understand crosslinker effect on the imprinted hydrogels loading and drug release behaviors for two extreme points.



6. STUDIES WITH TEGDMA AND IA WITH VARIOUS RATIOS

A combinatorial library had been developed and 5 selected factors which effect imprinted hydrogel performance had been discussed. It was concluded that hydrogels using IA and TEGDMA were more effective in doxycycline imprinting. The results of the analysis made with the response optimizer showed that, TEGDMA hydrogels showed outstanding drug released performance with IA. For this reason, it was decided to investigate the imprinting properties of 5% and 1% TEGDMA hydrogels, in detail.

6.1 Low Crosslinked IA-TEGDMA Based DOX- Imprinted Hydrogels

In this part, low crosslinked DOX-imprinted hydrogels was synthesized with HEMA, TEGDMA, and IA by thermal free radicalic polymerization.

Table 6.1 : Formulas applied in the synthesis of hydrogels.

Code	IA	TEGDMA	DOX	DOX:IA	Loaded Drug	Released Drug	IF Value
	Mol %	Mol %	Mol %		$\mu\text{g DOX/mg hydrogel}$		
MIP 1	1	1	0.160	6.25	1.92	3.62	1.13
MIP 2	3	1	0.160	18.75	2.35	5.69	0.85
MIP 3	5	1	0.160	31.25	2.0	7.63	0.67
MIP 4	1	1	0.205	4.88	1.42	3.06	0.83
MIP 5	3	1	0.205	14.63	2.56	5.12	0.96
MIP 6	5	1	0.205	24.39	1.94	5.20	0.62
MIP 7	1	1	0.250	4	1.78	4.67	1.04
MIP 8	3	1	0.250	12	2.70	5.70	0.98
MIP 9	5	1	0.250	20	1.34	3.34	0.43
NIP 1	1	1	-	-	1.70	3.67	
NIP 2	3	1	-	-	2.76	5.75	
NIP 3	5	1	-	-	3.13	6.76	

Numerous hydrogels were prepared with different molar ratio of DOX (0.160, 0.205, 0.250 mol %) and IA (1, 3, 5 mol %), keeping the proportions of the crosslinker constant as 1mol % in the absence of DOX. The thickness of the fabricated hydrogels attempted to be 450 μm .

The compositions and assigned codes of the polymer samples are given in Table 6.1. The fabrication of the hydrogels were completed as mentioned before. Drug loading capacity and release profile were compared with the corresponding non-imprinted hydrogel.

6.1.1 FTIR Analysis

For all hydrogels, the completion of the polymerization was monitored by FTIR spectrophotometer from characteristic unreacted C=C stretching double bond signals at 1650 cm^{-1} . An example of the spectra obtained after polymerization is given in Figure 6.1. The results showed that hydrogels have weak C=C double bond peak. It was indicated that, 100% conversion of monomers could not be reached after polymerization.

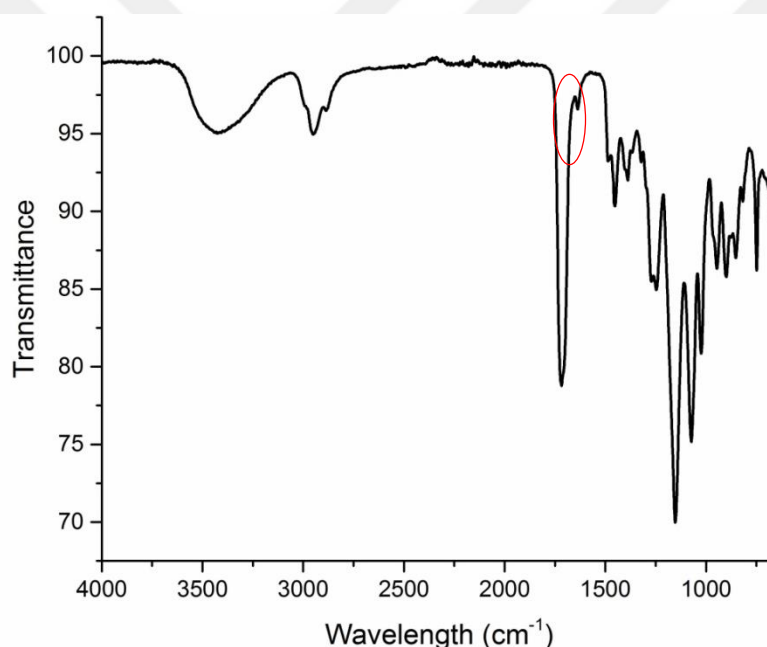


Figure 6.1 : ATR-FTIR spectra of PHEMA hydrogel (1IA-0.160) after polymerization.

6.1.2 Swelling analysis

After washing step, the imprinted and non-imprinted hydrogels were transparent, and exhibited similar appearance and elastic properties. Figure 6.2 a and b shows the effect of hydrogel composition in the feed on the swelling ratio. Specifically, the swelling ratio was slightly increased when monomer ratio increased from 1 mol % to 5 mol % (from 33.37 to 34.46 %) in the loading solution. There was not a significant effect of imprinting on the swelling ratios of 1 mol % and 3 mol % IA containing hydrogels. When the amount of monomer was increased to 5 mol % and the template molecule

ratio was raised up to 0.160 mol %, the swelling ratio dropped off slightly from 34.46 to 33.03 % in the loading solution. It can be said that this was not a significant decrease in the swelling ratio with imprinting. The swelling ratios in the 0.9 % NaCl solution were higher than those measured in the loading solution. Increasing monomer ratio for non-imprinted hydrogels in the releasing medium caused an increase in the swelling ratio from 48.85 to 56.25 % . While using a high amount of monomer ratio, increasing template ratio from 0.160 mol % to 0.205 mol % caused a decrease in swelling ratio from 56.58 % (NIP 3) to 53.85 % (MIP 6) in the releasing medium.

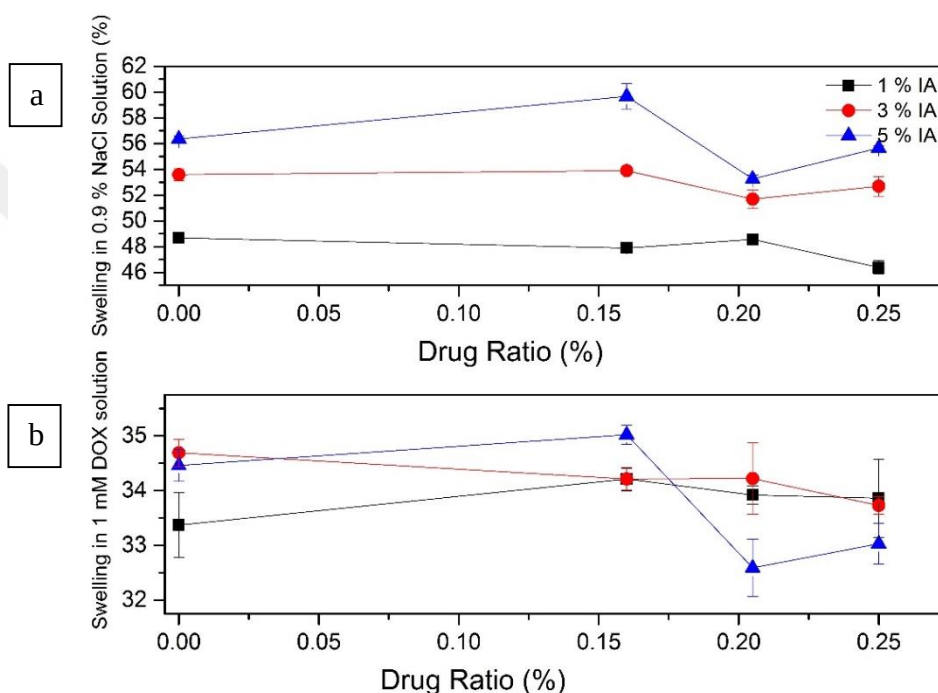


Figure 6.2 : Swelling ratios of the hydrogels in a. releasing medium b. loading solution.

In all cases, increased pH with changing swelling medium from loading solution to releasing medium led to an increase in swelling ratio of all hydrogels. The pH increased resulted in deprotonation of the carboxylic acid groups, electrostatic repulsion and increased hydrophilicity leading to hydrogel swelling. The concentration of the ionized groups led to an increase of swelling ratio with increasing monomer ratio. On the other hand, no major differences were detected in the swelling ratio of the network in the loading solution. We explained that IA were protonated in the loading solution which pH was higher than pKa values of IA in the hydrogel structure. The protonated IA may limit the hydrophilicity of the hydrogels. The swelling ratio decreased with imprinting when high monomer ratio was used to prepare hydrogels.

The organization of the functional monomer in the imprinted cavities led to a small decrease in the swelling ratio.

6.1.3 Loading studies

The dried hydrogels were immersed into the drug solution at pH 3.9 for loading studies. The hydrogels were neutral form in the loading solution and DOX was available in the solution as both cationic (DOXH_3^+) and zwitterion (DOXH_2^0) forms. As expected, increasing IA moiety increased loading capacity of the non-imprinted hydrogels. The presence of IA itself remarkably enhanced the affinity of the hydrogels for DOX. The effect of IA in the DOX-loading capacity of the hydrogels can be observed as hydrogen bonding between the functional groups of DOX and IA. The functional monomer ratio was found to have a significant effect on the loading behavior of the hydrogels depending on the imprinting ratio (M:T).

Figure 6.3, summarizes the amounts of DOX loaded by the imprinted and non-imprinted hydrogels prepared with different formulations as a function of monomer ratio and drug ratio presence in the polymerization. The template ratio was varied as 0.160, 0.205 and 0.250 % to explain the affinity properties of low crosslinked imprinted hydrogels. The amount of DOX loaded by imprinted hydrogels reduced with increasing monomer ratio from 1 mol % to 5 mol %. At low monomer ratio (1 mol % and 3 mol %), the amount of loaded DOX was very slightly greater. Therefore, there was not a significant difference between the imprinted and non-imprinted hydrogel while template ratio increased from 0.160 mol % to 0.250 mol %. However, loading for the hydrogels containing 5 mol % IA decreased obviously with increasing DOX ratio using in the polymerization, especially for MIP 9 in which M:T ratio was 20.

The loading capacity of the hydrogels was reduced dramatically when the IA moiety increased to 5 mol %. It could be attributed that higher amount of IA incorporated into the imprinted cavities. Consequently, adequate number of IA may arrange in the specified cavities, this allowed to interact of DOX and monomers from more number of functional sites. It resulted in a decrease in number of imprinted cavities but an increase in strength of binding to DOX. When there was a lack of the functional monomer, the cavities were created with the lack number of functional monomer, which this caused low binding affinity to DOX with uncompleted complexation. When there was insufficient amount of DOX in the polymerization mixture, the M:T ratio

was increased up to 31.25. In this case, there were 20 functional monomers in the imprinted cavities, and the rest was randomly settled. This case resulted in binding of DOX molecules by both the imprinted cavities and randomly distributed functional groups of IA. The difference between the imprinted and non-imprinted hydrogels decreased or disappeared. Studies with IA and TEGDMA had shown that the low imprinting factor is acceptable for successful imprinting and made positive impact on the releasing performances of the imprinted hydrogels.

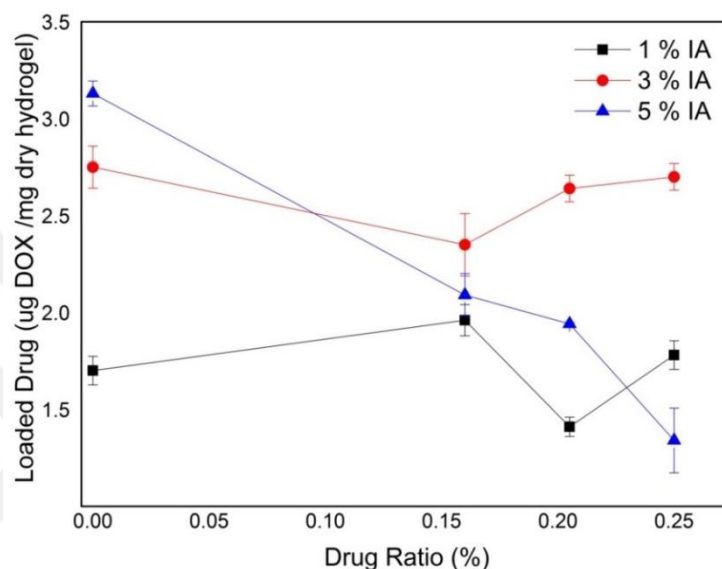


Figure 6.3 : a: The loading capacities of the hydrogels synthesized with different functional monomer ratios as a function of DOX ratio.

In a study which was published by Alvarez-Lorenzo et al. [25], they investigated the effect of M:T ratio on imprinting efficiency. Norfloxacin was used as template and AA was used as functional monomer. They indicated that M:T ratio was a critical variable due to creation of high binding affinity. They found that the number of functional monomers in the imprinted cavities increased with increasing template proportion in the polymerization and it may cause to a decrease in drug loading. In an another study, timolol was used as crosslinker and the loading capacity of the hydrogels decreased with decreasing template ratio presence in the polymerization [137]. Above or below of optimum ratio the hydrogels had higher or similar loading capacity with non-imprinted counterparts

On the other hand, the swelling behavior directly affected the drug loading and release behavior of the hydrogels which was also engaged with the structural parameters such as $V_{2,s}$, ξ and $\bar{M}_c$. Structural parameters of the hydrogels were poltted in Figure 6.4

and 6.5. The volume fraction in swollen state values were comparable for 5 mol % IA containing hydrogels when the monomer and drug ratio were varied in the loading solution, also hydrogels containing 1 and 3 mol % IA had nearly similar $V_{2,s}$ for all template ratios (Figure 6.4). The results for $\bar{M}_c$ showed that there was not a significant difference between imprinted and non-imprinted hydrogels for 1 and 3 mol % IA containing hydrogels in low template ratios. Addition of DOX as template into the pre-polymerization mixture with other components leads to create a hydrogel with higher molecular weight between two crosslinks ($\bar{M}_c$) than the non-imprinted hydrogels. On the contrary, the results showed that $\bar{M}_c$ decreased with increasing the drug moiety in pre-polymerization process when IA ratio was 5 mol % (Figure 6.5).

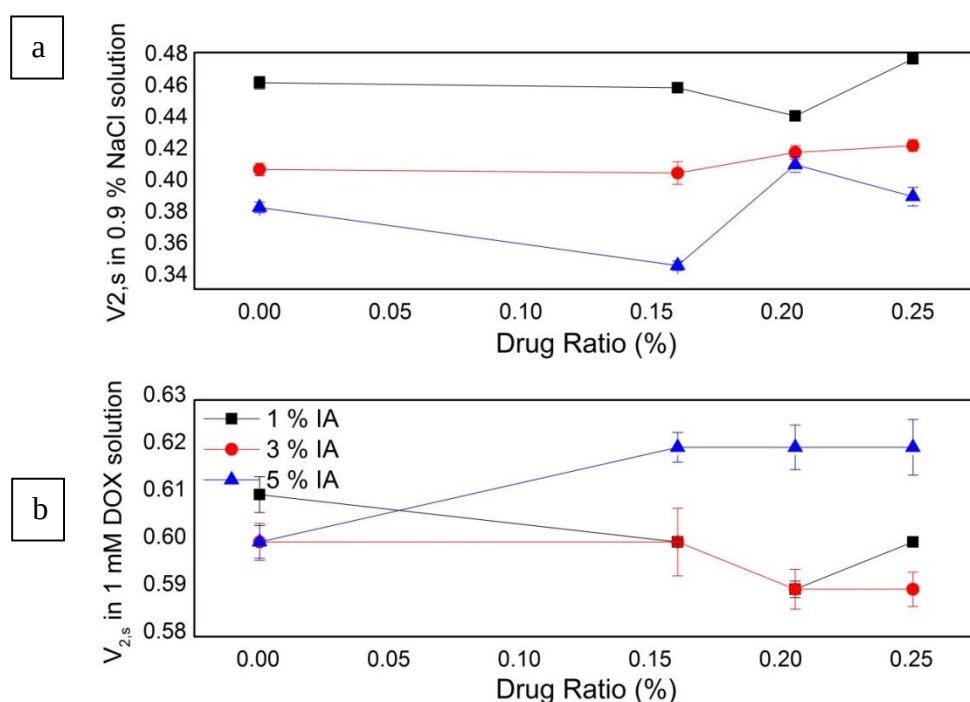


Figure 6.4 : $V_{2,s}$ values of MIPs and NIPs in loading solution and releasing medium.

It seems that DOX may influence the arrangement of the polymer chains and correlated crosslink structure in the hydrogel causing to a different $\bar{M}_c$, $V_{2,s}$ and ξ values related with the M:T ratio. It was expected that the loading capacity of the hydrogels considerably decreased with decreasing of the mesh size, which would influence the arrangement of functional groups interacting with the template. The loading capacity of the hydrogels decreased at high functional monomer ratio (5 mol %) and increased at low functional monomer ratio (1 mol %). It can be said that similar macromolecular architectures were not available for free volume transportation while the hydrogels imprinted with different M:T ratios for 5 mol % containing IA monomer. This can be

attributed to the mesh size and molecular weight between two crosslinks which decreased with molecular organization in imprinted hydrogel network at high monomer ratio with high template ratio presence in the polymerization. Lower $\bar{M}_c$ caused rigid structure, limited the swelling of the hydrogel, and prevented entrance of drug molecules into the hydrogel matrix.

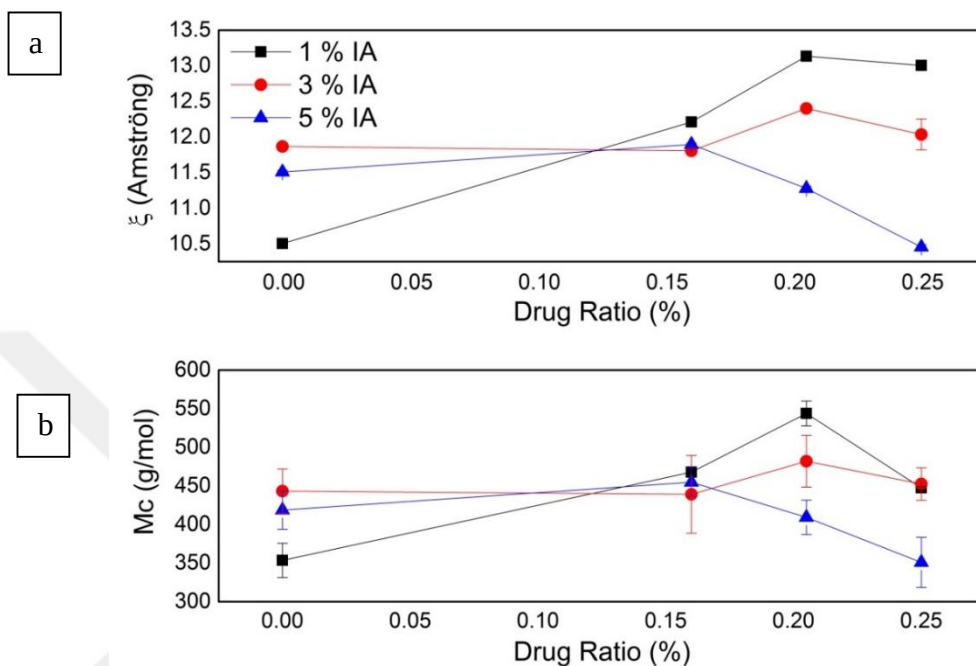


Figure 6.5 : $\bar{M}_c$ and ξ values change with functional monomer and drug ratio.

These results are in consistent with the results found in literature. Maryam Ali et al. showed that presence of the template in the pre-polymerization mixture changed crosslink structure of the hydrogel and increased the molecular weight between the two crosslinks of the hydrogels without functional monomer [26]. Addition of the functional monomer caused a decrease in $\bar{M}_c$ values. Therefore, we might conclude that increasing the functional monomer ratio led to increase the effect of imprinting with higher template ratios.

In the releasing medium, the structural changes were compared with using $V_{2,s}$ values as an indicator which were calculated from swelling results in the releasing medium. There was a noticeable decrease in $V_{2,s}$ values of the hydrogels by changing the swelling medium which was associated with carboxyl ion concentration. With increasing pH, the ionization induced and swelling ratio increased. The hydrogels expanded as a function of functional monomer and M:T ratio. At the same time, it caused an increase in mesh size as the swelling increased. Volume fraction in swollen

state decreased with increasing functional monomer ratio. The ionized carboxyl group concentration led to decrease of polymer volume in swollen state.

There was no significant effect of imprinting on $V_{2,s}$ values of 1 mol % and 3 mol % IA including NIPs and MIPs in the 0.9 % NaCl solution. However, $V_{2,s}$ for hydrogels containing 5 mol % IA changed obviously as the amount of template molecule increased. Specifically, It was seen that, NIP 3 had $V_{2,s} = 0.38$ value and imprinted hydrogels had 0.35, 0.41, 0.39 of $V_{2,s}$ in order of M:T ratio (M:T 32, 24.4, 20). The addition of DOX as template which was used for functional monomer template complexation in pre-polymerization mixture, may make a noticeable difference in releasing profiles of MIPs containing equal amounts of monomer by changing affinity of binding sites and volume fraction in swollen state due to the changes in pH of swelling medium.

In the releasing medium, pH increased to 7.00 which was above the Pka_1 and Pka_2 values of IA and Pka_1 values of DOX. The rise in pH led to deprotonate of carboxylic groups of IA and zwitterionic formation of DOX. Therefore, charge repulsion occurred as a consequence of the destruction of the hydrogen bonds between DOX and IA. With deprotonation of the IA functional groups, swelling degree increased and hydrogen bond between template and functional groups may convert to electrostatic interaction. And also, increasing mesh size and decreasing volume fraction in swollen state led to decrease the bond strength due to increase distance between monomer and drug molecule. All these effects finally led to the release of drug.

6.1.4 Drug release studies

When the releasing profiles of the MIPs and NIPs were examined, no direct connection was found with loading characteristics. This situation may relate to the variation of volume fraction of the hydrogels in swollen state and mesh size via pH differences of the release and loading environment. Since DOX and IA were in different charge states in both environments, interactions between the DOX and hydrogel changed due to charge concentration varied with functional monomer concentration. From the release profile presented at Figure 6.6 (a-c), it was clear that drug release pattern varied with both monomer and M:T ratios. Namely, MIP 3 and MIP 7 hydrogels released more amount of DOX than their counterparts. The amount of released DOX decreased with increasing DOX ratio presence in the polymerization mixture (decreasing M:T ratio

from 31.25 to 20) for 5 mol % IA containing hydrogels. Hydrogels containing 3 mol % IA exhibited similar releasing pattern when the amount of released DOX was compared with each other. MIP 4 released less amount of DOX which had 24.4 of M:T ratio and MIP 7 released more amount of DOX which had 31.25 of M:T ratio than NIP 3. Additionally, it was observed that MIP 3 had the lowest $V_{2,s}$ value in the releasing medium and highest $V_{2,s}$ value in loading medium.

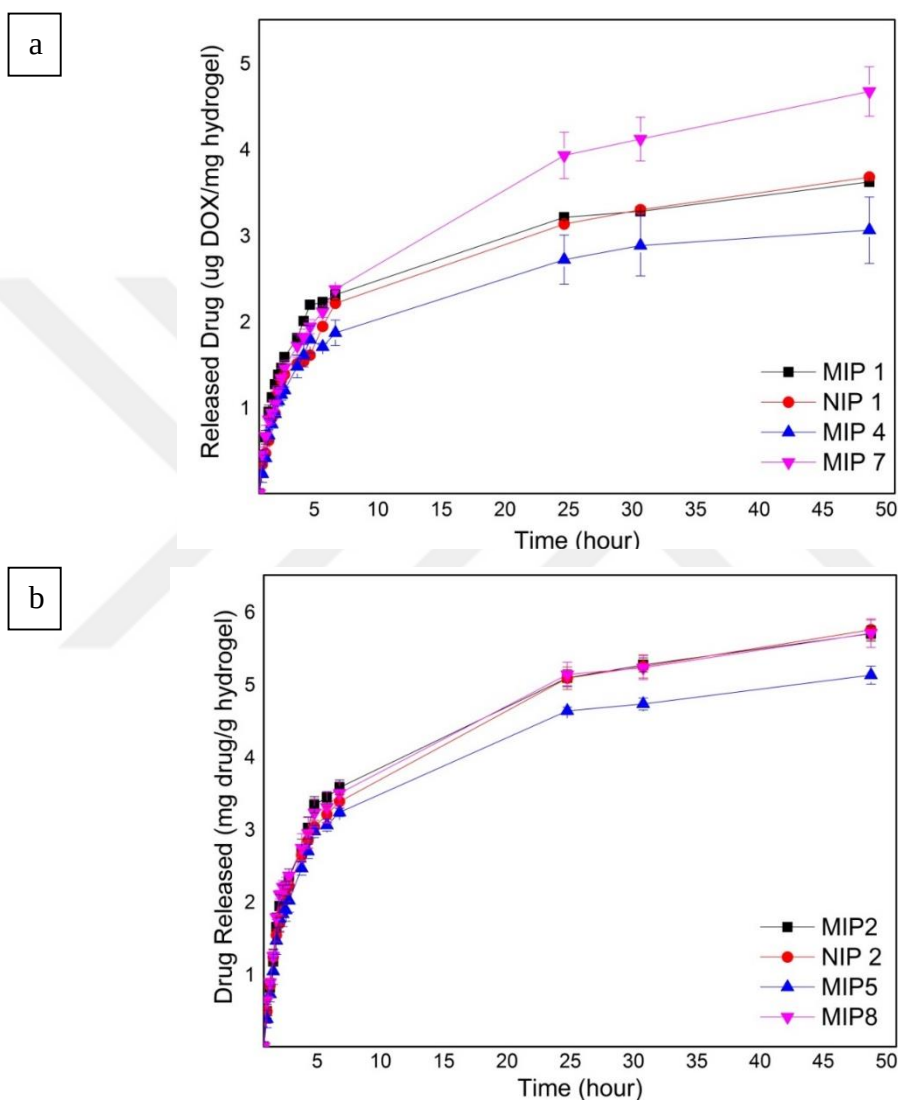


Figure 6.6 : DOXH amount of release profiles from PHEMA hydrogels prepared with 1% TEGDMA and different functional monomer and DOXH ratio used to prepare imprinted pHEMA hydrogels on their ability to release in 0.9% NaCl solution. a:1% b:3% c:5% IA.

c

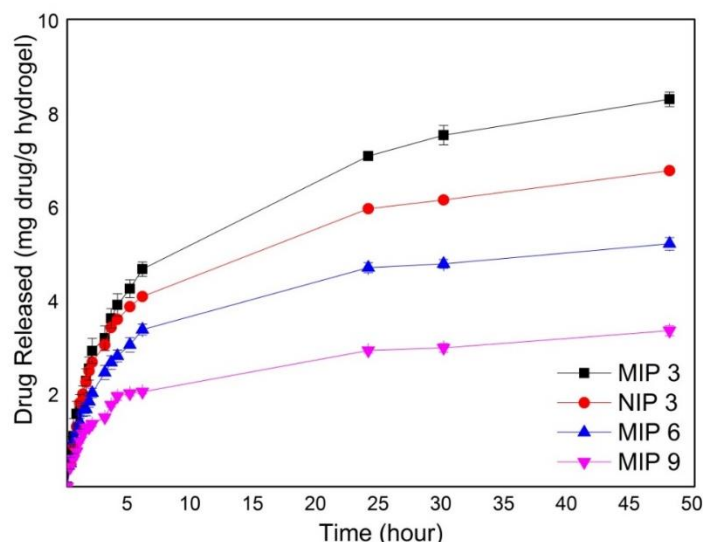


Figure 6.6 (Continued): DOXH amount of release profiles from PHEMA hydrogels prepared with 1 mol % TEGDMA and different functional monomer and DOXH ratio used to prepare imprinted pHEMA hydrogels on their ability to release in 0.9 % NaCl solution. a:1 mol % b:3 mol % c:5 mol % IA.

6.1.5 Mathematical modelling of drug release behavior

Figure 6.7 (a-c) represented cumulative percentage of DOX releasing from all hydrogels as a function of time. Generally, 50-70 % drug released in 6 hours for all hydrogels depending on the composition and imprinting. In this study, drug released fastly at the first phase and relatively slow at the second phase. In the third phase, drug released very slowly.

As it can be seen from Figure 6.6 a and Figure 6.7 a, there were a relationship between cumulative release and cumulative percentage behaviors of the hydrogels. The MIP 3 and MIP 7 hydrogels released the higher amount of DOX and also released the lower percentage DOX in 6 hours than their counterparts. When IA ratio was 3 mol %, cumulative percentage in 6 hours for non-imprinted and imprinted hydrogels were similar.

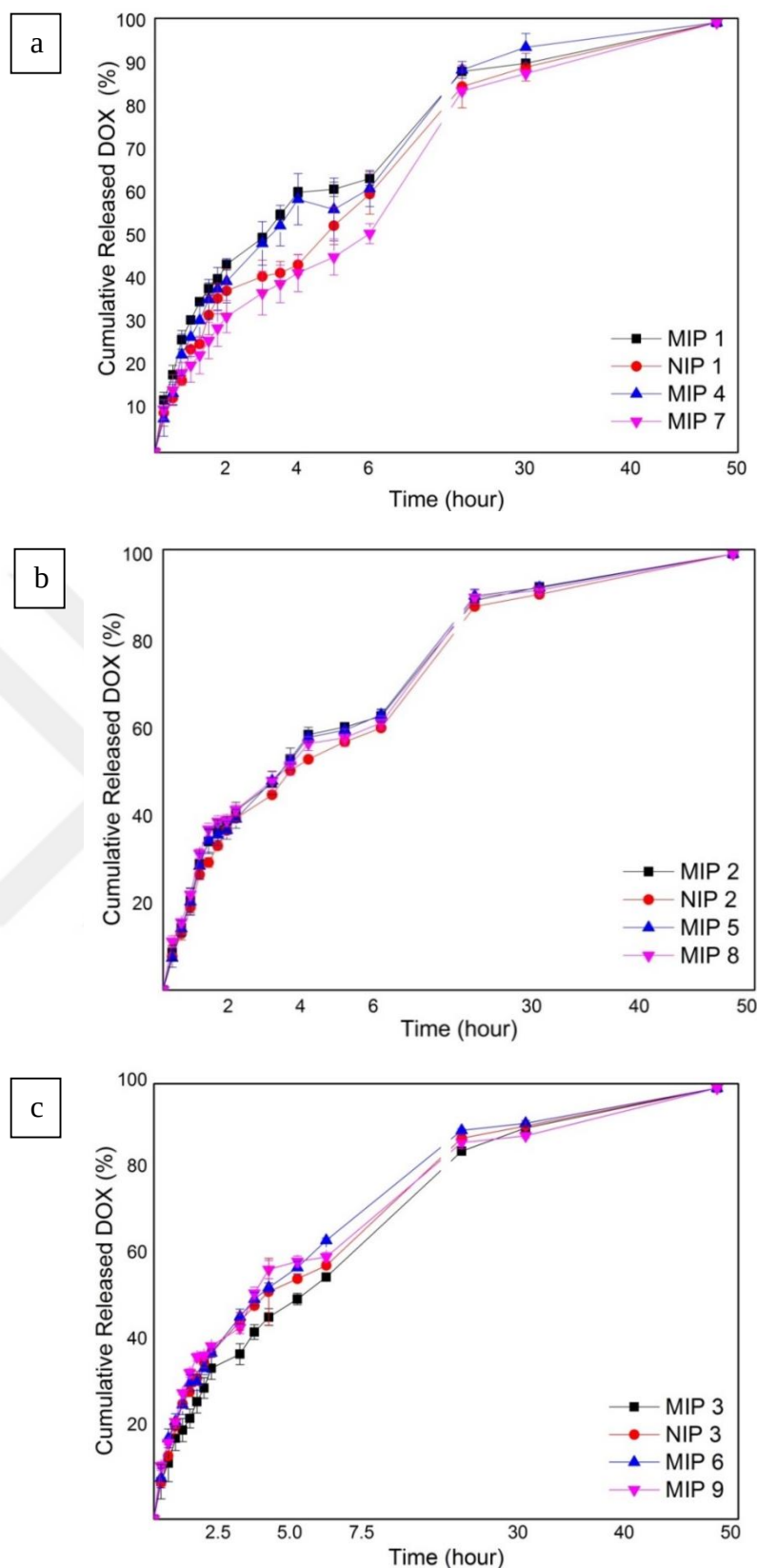


Figure 6.7 : DOX cumulative release profiles from PHEMA hydrogels prepared with 1 mol % TEGDMA and different functional monomer and DOX ratio used to prepare imprinted pHEMA hydrogels on their ability to release in 0.9% NaCl solution. a:1 mol % b:3mol % c:5 mol %.

In order to evaluate the effect of the hydrogel composition and the imprinting on the drug transport mechanism, Korsmeyer Peppas approach was used. Regression coefficients showed that $M_t/M_\infty \sqrt{t}$ curves were linear up to a fractional release of 0.6 for all hydrogel formulations, and that the slope was dependent on the hydrogel composition and the template ratio presence in the polymerization.

In order to gain insight into the releasing mechanism, n values calculated from Korsmeyer-Peppas Equation and presented in Table 6.2. The hydrogels released DOX in a biphasic releasing pattern. All the formulations exhibited good linearity ($R^2 \geq 0.98$) for both phases. At the first phase, n values were $0.43 < n < 0.89$ (except for MIP 5), indicating that DOX releasing took place via non-Fickian diffusion mechanism. Drug release were controlled by both diffusion and chain relaxation resulting in swelling. However, the imprinting did not change the releasing transport mechanism, the balance between diffusion and swelling may change. Swelling and diffusion properties investigated to gain a depth insight into imprinting effect. The swelling results showed that similar swelling properties were exhibited for 1 and 3 mol % IA containing hydrogels. There was only a 2 % decrease with the imprinting of 5 mol % IA containing hydrogels.

Table 6.2 : Korsmeyer Peppas n values and regression coefficients of the NIPs and MIPs.

Code	n ₁	R ²	n ₂	R ²
MIP 1	0.676	0.993	0.423	0.988
MIP 2	0.849	0.992	0.455	0.977
MIP 3	0.631	0.988	0.470	0.981
MIP 4	0.856	0.990	0.500	0.991
MIP 5	0.960	0.998	0.475	0.984
MIP 6	0.617	0.991	0.512	0.976
MIP 7	0.519	0.995	0.465	0.995
MIP 8	0.748	0.974	0.399	0.982
MIP 9	0.720	0.978	0.484	0.995
NIP 1	0.720	0.980	0.588	0.989
NIP 2	0.775	0.991	0.402	0.995
NIP 3	0.846	0.993	0.410	0.990

The diffusion coefficient, change in volume fraction of hydrogels in swollen state values and n values obtained from Korsmeyer Peppas equation proved that imprinting was an important process in generation of high affinity and well organized binding

sites. The results proved that imprinting not only related with monomer-template interaction but also affect nanostructure and diffusion behavior of the hydrogels.

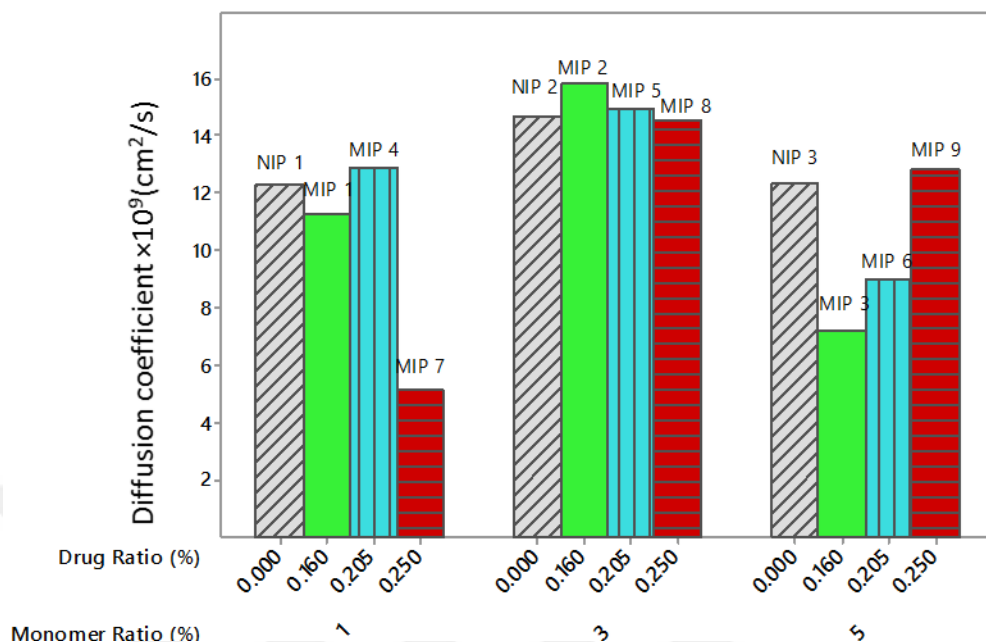


Figure 6.8 : Diffusion coefficient of the hydrogels prepared with different monomer and template ratios.

The diffusion coefficient was calculated from the slope of the $M_t/M_\infty - \sqrt{t/L^2}$ curve. The results indicated that the hydrogel matrix slowed down the DOX diffusion (Figure 6.8). MIP 3 and MIP 7 had the lowest diffusion coefficients. Although, imprinting influenced on diffusion behavior of low crosslinked hydrogels, DOX diffusion was not influenced effectively by the functional monomer ratio. Increasing diffusion path length with lower volume fraction resulting from the increase in the hydrogel volume caused a slower release kinetics.

It can be seen from Figure 6.8 that the moderate increase in hydrogel swelling of these hydrogels did not affect the rate of DOX release. The diffusion coefficients of the hydrogels containing 1 and 3 mol % IA were not effectively changed with imprinting; except for MIP 3. 5 mol % IA containing hydrogels had notably different diffusion coefficients with applying imprinting. While M:T ratio increased from 20 to 31.25, diffusion coefficient decreased from 12.4×10^{-9} to 7.2×10^{-9} cm²/s. This case related to arrangement of the functional monomer in the cavities with high affinity and increasing of randomly distributed IA in the hydrogel matrix. MIP 3 and MIP 7 had the lowest diffusion coefficients. This can be attributed to the drug release control which was dominated by diffusion with successful imprinting. When all the obtained

results were evaluated together, MIP 3 as an imprinted hydrogel appeared to be the more controlled DOX release than other both imprinted and non-imprinted hydrogels.

Besides, using low amount of crosslinker greatly reduced the success of molecular imprinting. We can show reasons by the fact that the mesh size was generally above the template radius, the interaction between the monomer and the drug was not sufficiently established. On the other hand, drug diffusion delayed with binding site interactions through the diffusion path.

6.2 High Crosslinked Molecular Imprinted Hydrogel Performances

High crosslinked (5 mol % TEGDMA) DOX-imprinted hydrogels was prepared with 2-Hydroxyethyl methacrylate (HEMA), triethyleneglycol dimethacrylate (TEGDMA), itaconic acid (IA) by thermal free radicalic polymerization. The ratio of the crosslinker was kept constant and twelve hydrogels were prepared with different molar ratio of DOX (0.160, 0.205, 0.250 mol %) and IA (1, 3, 5 mol %), and also in the absence of DOX. The thickness of the fabricated hydrogels were 170 μm .

Table 6.3 presented the assigned code and the compositions of the hydrogels. The synthesis method of the hydrogels were the same as previous ones. Drug loading capacity and release profile were compared with the ones of the corresponding non-imprinted hydrogel.

Table 6.3 : Hydrogel codes for imprinted and non-imprinted hydrogels.

Code	IA Mol %	TEGDMA Mol %	DOX Mol %	DOX:IA	Loaded Drug μg DOX/ mg hydrogel	Released Drug μg DOX/ mg hydrogel	IF Value
MIP 1	1	5	0.160	6.25	2.78	2.01	1.65
MIP 2	3	5	0.160	18.75	1.88	3.77	1.19
MIP 3	5	5	0.160	31.25	2.66	2.25	1.05
MIP 4	1	5	0.205	4.88	2.28	2.08	0.90
MIP 5	3	5	0.205	14.63	2.28	4.79	1.45
MIP 6	5	5	0.205	24.39	2.24	2.69	0.91
MIP 7	1	5	0.250	4	3.33	2.51	1.91
MIP 8	3	5	0.250	12	3.68	3.13	2.04
MIP 9	5	5	0.250	20	4.32	4.68	1.88
NIP 1	1	5	-	-	1.70	2.16	
NIP 2	3	5	-	-	1.58	2.25	
NIP 3	5	5	-	-	2.55	2.55	

6.2.1 FTIR studies

FTIR spectrophotometer was used to determine characteristic unreacted C=C stretching double bond signals at 1650 cm^{-1} . Figure 6.9 represented a spectra example monitored after polymerization before washing. There was a weak C=C double bond peak after polymerization indicating 100% conversion of monomers could not be reached.

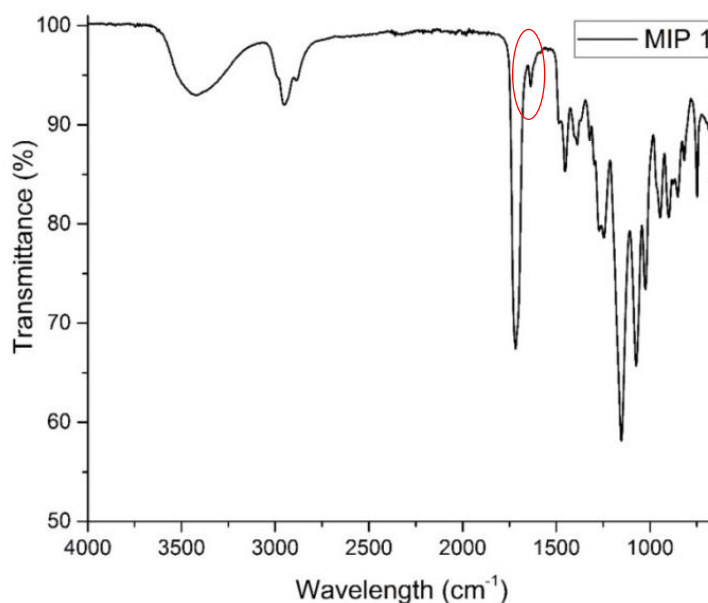


Figure 6.9 : ATR-FTIR spectra of PHEMA hydrogel (1IA-0.160) after polymerization.

6.2.2 Swelling studies

After washing step, the imprinted and non-imprinted hydrogels were transparent, and exhibited similar appearance as the former ones. The swelling ratio of the hydrogels network was determined in similar conditions as applied in the drug release experiments. The water absorption capacities of the hydrogels were presented in Figure 6.10.

It can be seen that the swelling values in the loading medium were not significantly influenced by the amount of the monomer. Furthermore, hydrogel swelling increased by 1-3 weight % after raising the monomer ratio from 1 to 5 mol % in the loading solution. The hydrogels continued to swell in the release environment due to the deprotonation of the carboxyl groups that caused to electrostatic repulsion. Similar to the results found in the literature [231], it was seen that swelling increased as a function of the carboxyl concentration. The differences in swelling ratio grew and 10 % weight

differences occurred between 1 mol % IA containing hydrogels and 5 mol % IA containing ones in the releasing medium. For all functional monomer ratios, no obvious difference was found with increasing drug ratio in the prepolymerization mixture. The swelling ratio decreased when the crosslinker ratio increased from 1 to 5 mol % as expected (Figure 6.2 and 6.10). Furthermore, more ratio of crosslinker prevented the water molecules from entering to the hydrogel matrix, resulting in less swelling ratio.

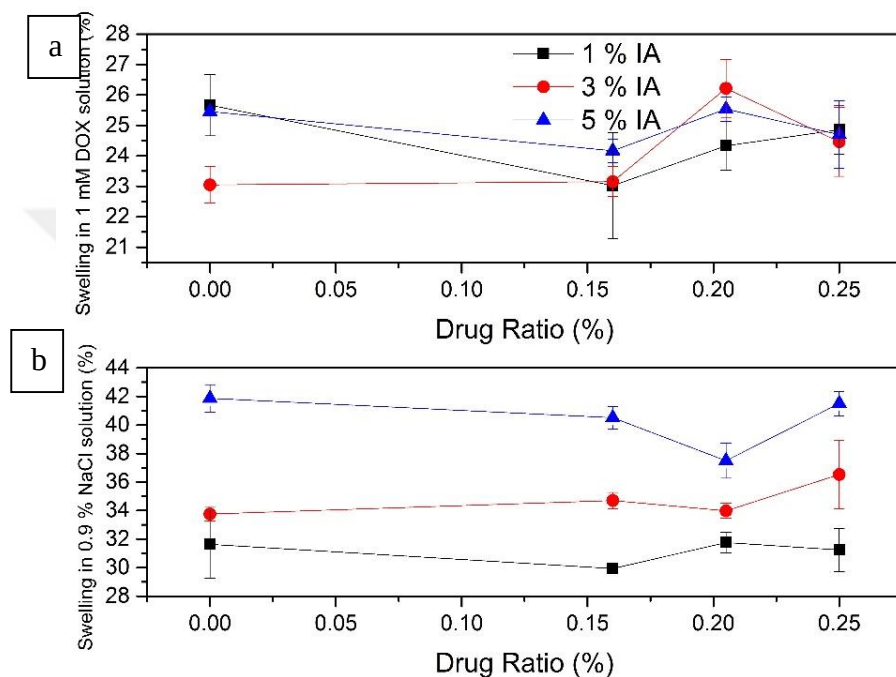


Figure 6.10 : Swelling ratios of the hydrogels in loading (a) and releasing mediums.

6.2.3 Loading studies

Loading capacities of both imprinted and non-imprinted hydrogels were plotted in Figure 6.11. Here, IA was in protonated state below the Pka_1 value of the ionizable groups [248]. Moreover, DOX was available in the solution as both cationic ($DOXH_3^+$) and zwitterion ($DOXH_2^0$) forms. Therefore, hydrogen bonding and electrostatic interaction played important role on the DOX binding ability of the hydrogels.

The loading capacity of the hydrogels was summarized in Table 6.3. The template ratio was increased from 0.160 to 0.250 mol % to explain the interactions of template to crosslinker and monomer. Amount of loaded DOX increased by increasing the IA ratio (from 1.70 to 2.55 $\mu\text{g DOX/mg hydrogel}$) for the non-imprinted hydrogels. Loading capacity of the imprinted hydrogels increased by increasing template proportion presence in the polymerization from 0 to 0.250 mol %. At low template proportion

(0.160 mol % and 0.205 mol %), the amount of loaded DOX increased very small. It can be said that there was a slightly difference between the imprinted and non-imprinted hydrogel while template ratio increased from 0.160 mol % to 0.250 mol % (except for MIP 1 because it had high IF value which was 1.65). However, loading capacity noticeably enhanced with using 0.250 mol % template for all monomer ratios, specifically MIP 8 had the highest IF value which had 3 mol % IA and 0.250 mol % template through the course of polymerization.

Loading capacity changed as a function of monomer ratio and M:T as plotted in Figure 6.11. In the preparation of non-covalent MIPs, the crosslinker ratio was kept constant as 5 mol % ratio. We can expect that there should be an optimal X:T ratio by holding all other parameters constant. Namely, when X:T was equal to 20 (MIP 7-8-9), the amount of loaded drug into the hydrogel increased by increasing the monomer ratio. It could be attributed to higher amount of template was responsible for generation of large number of the imprinted cavities. Sufficient number of IA may arrange around these template molecules and this allow to interact of DOX and monomers from more number of functional sites. It may result in an increase in number of imprinted cavities with high crosslinked ratio and also an increase in strength of binding to DOX. When there was a lack of the template molecules (hydrogels which had high X:T ratio), excess number of cavities created with randomly distributed functional monomers and this caused low binding affinity to DOX due to IA-TEGDMA and DOX-TEGDMA interactions. When monomer ratio was increased from 1 to 5 mol % the loading capacity increased 2.51 to 4.68 μg DX/mg hydrogel (from MIP 7 to MIP 9). In this situation, the number of functional monomer increased in the imprinted cavities. These cavities may be enough to arrange the functional monomers with sufficient number of template. This case resulted in the binding of DOX molecules by the imprinted cavities with a strong affinity. The difference between the imprinted and non-imprinted hydrogels pronounced and IF value became greater than 1.

The employment of a higher template moiety during polymerization increased monomer-template interaction in the hydrogel network which had highly crosslinked structure; resulting in a hydrogel with maximum number of binding sites while maintaining sufficient rigidity to protect the integrity of the imprinted cavities. When X:T ratio was above 20 (X:T= 24 or 31.25), the effect of imprinting gradually diminished with decreasing template ratio due to a decrease in IA-DOX interaction

and an increase in IA-TEGDMA interaction. It has been noticed that the binding affinity of the cavities were affected by the crosslinker: template ratio (X:T). It is likely that the crosslinker played a crucial role in determination of the structure and sizes of the resulting cavities.

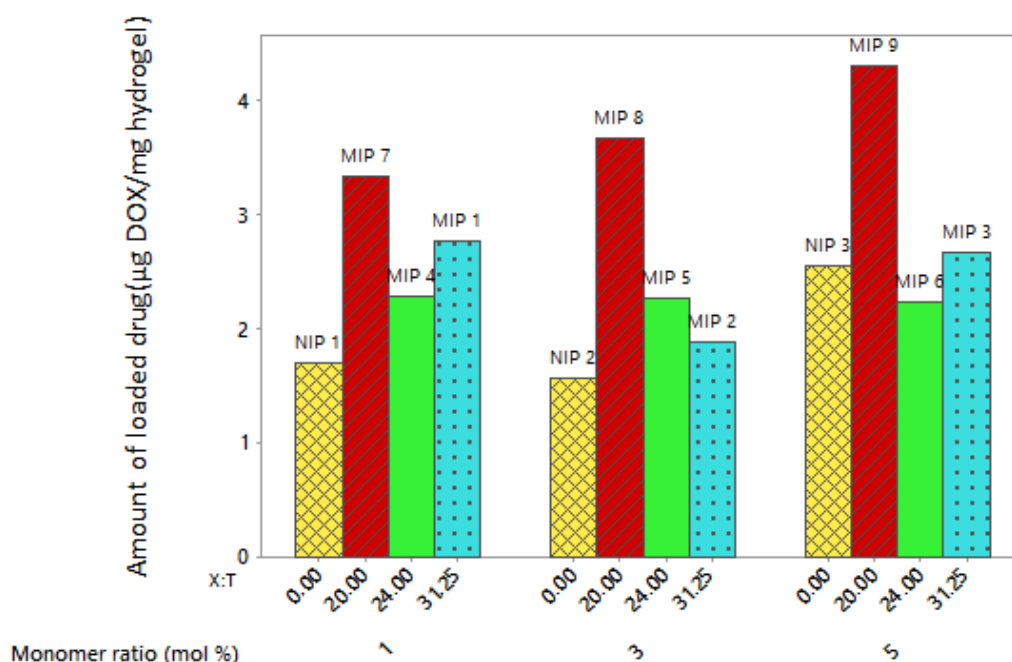


Figure 6.11 : Drug loading capacities of the hydrogels.

Therefore, selection of the crosslinker ratio greatly affected the formation and destruction of binding sites, and was of great importance in the design of a MIP. In a study that investigated the effect of crosslinker on monomer and template interaction, it was demonstrated that imprinting was more efficient in a certain crosslinker ratio which led to the highest interaction between functional monomer (AA) and doxycycline [167]. It means that not only M:T ratio but also X:T and monomer ratio presence in the polymerization were notably important for a successful imprinting.

Figure 6.12 presented volume fraction in the swollen state of the hydrogels in loading solution and releasing medium, respectively. The $V_{2,s}$ values decreased with changing medium from loading to releasing. The $V_{2,s}$ value of non-imprinted hydrogels in the loading solution was between about 0.70 and 0.72 and the effect of functional monomer was very small. There was a very small effect of imprinting on the volume fraction of the hydrogels. As the amount of template presence in the polymerization mixture increased, 2% change in $V_{2,s}$ value was observed. When 0.250 mol % template was used to prepare imprinted hydrogel, all imprinted hydrogels had similar volume fraction where the X:T ratio was equal to 20. $V_{2,s}$ values dropped due to swelling of

the hydrogels in the release medium. The $V_{2,s}$ were decreased in order of NIP 1, NIP 2, NIP 3 depending on the amount of IA in the structure. The same trend was valid for the imprinted hydrogels. The change in $V_{2,s}$ was notable for the imprinted hydrogels containing 5 mol % IA. $V_{2,s}$ increased from 0.50 to 0.55 with increasing drug ratio presence in the polymerization (from NIP 3 to MIP 9).

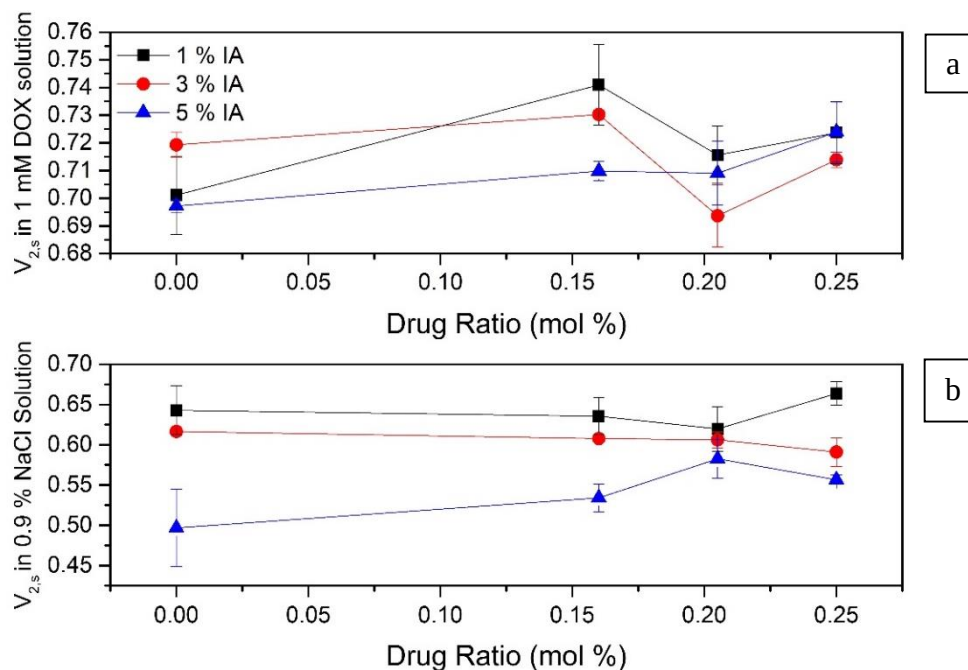


Figure 6.12 : Volume fraction of the polymers in swollen state as a function of drug ratio presence in the polymerization.

These results suggested that DOX loading can be controlled via the specific binding sites within the polymer network. Loading difference was significantly increased from the non-imprinted hydrogels compared to imprinted hydrogels despite having very similar structures as determined experimentally. It was obvious that imprinting affected DOX loading capacity compared to non-imprinted counterparts and was not due to structural properties.

Figure 6.13 represented the molecular weight between two crosslinks and mesh size of both imprinted and non-imprinted hydrogels. The addition of DOX as template to the pre-polymerization mixture with other components led to creation of a hydrogel with slightly lower molecular weight between two crosslinks ($\bar{M}_c$) and mesh size than the non-imprinted hydrogels. Molecular weight between the two crosslinks and mesh size got closer to each other for different monomer ratios with increasing drug ratio.

While drug ratio increased from 0.160 to 0.250 mol % the structure of the hydrogels suited similar to each other and the hydrogels had X:T ratio of to 20.

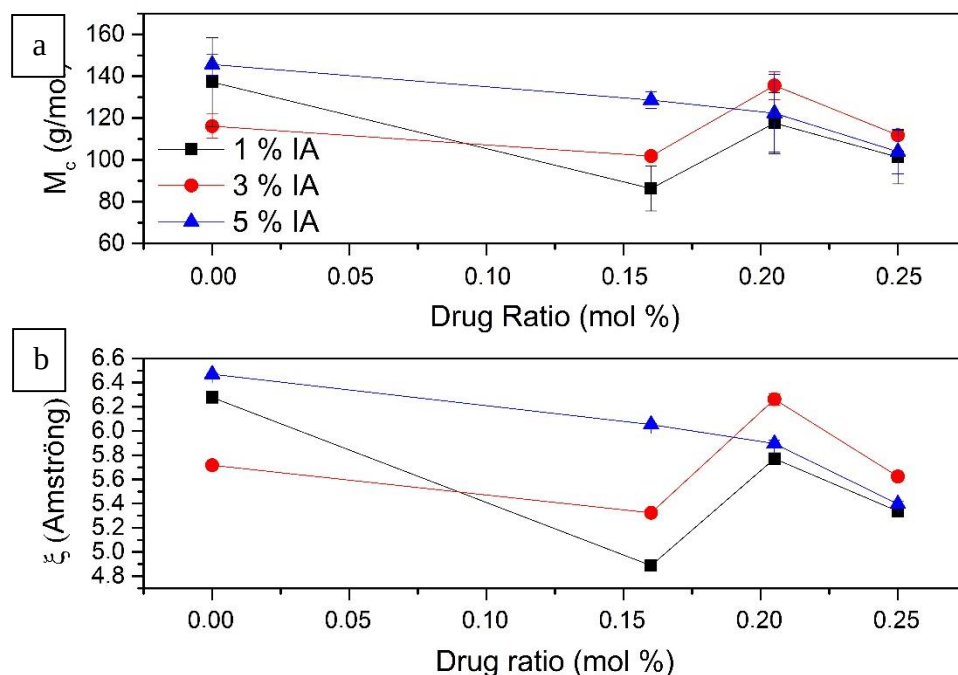


Figure 6.13 : Structural parameters as a function of drug ratio reside in the polymerization.

The imprinted hydrogels in general had much greater loading (2 times more) than non-imprinted counterparts at approximately similar volume fraction in the swollen state and at slightly smaller average molecular weight between crosslinks and mesh size. This may be explained by the fact that a more organized structure was established by imprinting.

6.2.4 Drug release studies

Volume fraction in the swollen state and diffusion with functional monomer-drug molecule interactions were the main parameters on determination of the releasing properties of the hydrogels. The binding sites affinity and alignment of monomers around the template changed the structural parameters (ξ and $\bar{M}_c$) and also diffusion properties of the hydrogel. As expected, the higher crosslinker ratio led to a great reduction in the amount of release. The effect of high crosslinker ratio can be described by mesh size reduction of the hydrogel matrix. High cross-linked polymers were less acidic as the carboxylate groups were hidden due to the high crosslinker ratio decrease the ionization. The relaxation of the polymer chains was decreased

responsible for lower swelling of the hydrogel. These results were in good agreement with other published results by Syed Majid Hanif Bukhari, and Shah et al. [161].

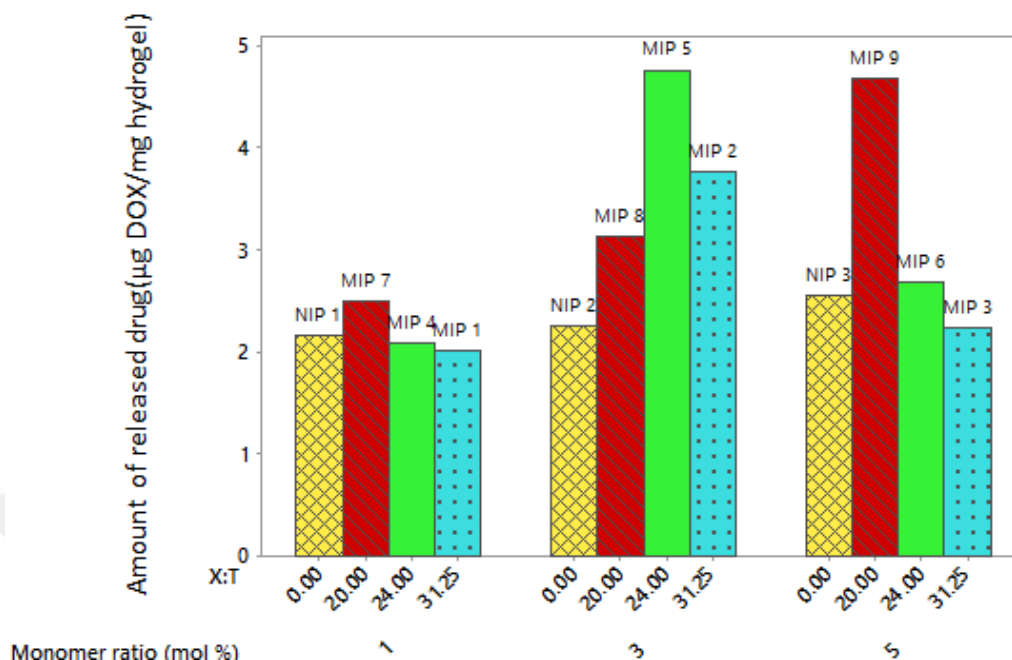


Figure 6.14 : Amount of released DOX as a function of M:X and X:T ratios.

The effect of monomer and X:T ratios on the total amount of released drug from hydrogels were summarized in Figures 6.14 and 6.15. According to the results, the monomer ratio the hydrogels exhibited different behaviors with change in X:T ratio. When monomer ratio was equal to 1 mol %, all hydrogels released nearly the same amount of DOX. However, when the monomer ratio increased to 3 mol % ratio all imprinted hydrogels released more drug than non-imprinted control hydrogel. The cumulative released DOX increased, until X:T was equal to 24. Finally, at 5 mol % ratio only MIP 9 released more drug than the non-imprinted ones which had X:T ratio of 20. MIP 3, which had excess monomer and insufficient amount of template presence in the polymerization, released lower amount of DOX than non-imprinted counterpart (NIP 3). When the monomer ratio was 1 mol % (M:X was equal to 0.2), less drug may be released due to the irreversible binding as the ratio of X:T increased from 20 to 31.25 (drug ratio decreased from 0.250 to 0.160 mol %).

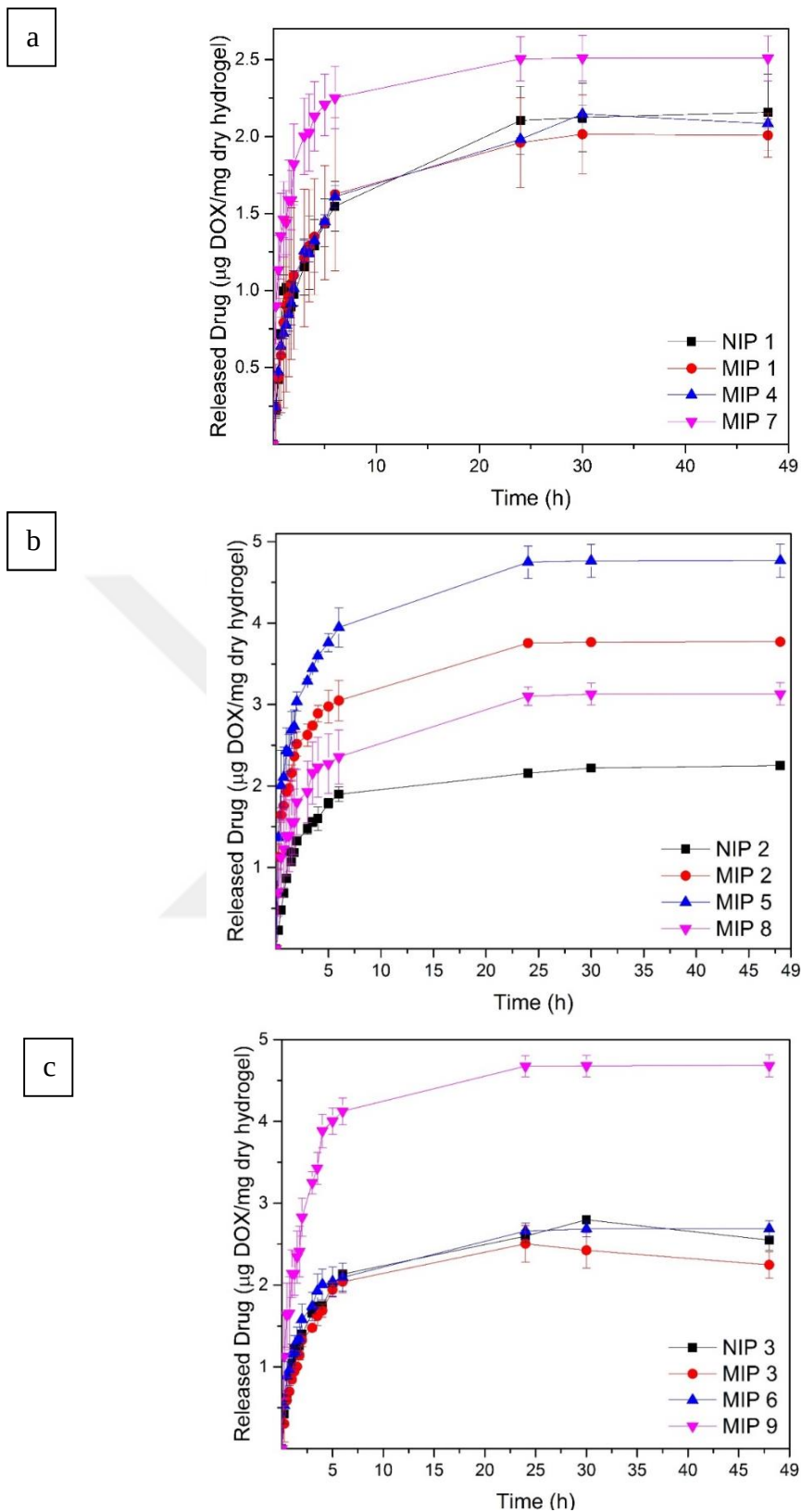


Figure 6.15 : Drug release patterns of the both imprinted and non-imprinted hydrogels.

6.2.4 Mathematical modelling of drug release

From the results presented in Figure 6.16, releasing of the DOX from highly crosslinked hydrogels took place by different types of transport mechanism related with the hydrogel composition. This can be described by the fact that exponent in “n” values higher than 0.89 for NIP 1 and NIP 2 governed by super case II transport and between 0.45 and 0.89 governed by non-Fickian diffusion for NIP 3, MIP 1, MIP 3 and MIP 4. The remaining with “n” values near and below 0.45 indicated Fickian transport for NIP 3, MIP 2, MIP 5, MIP 6, MIP 7, MIP 8 and MIP 9. In general, when monomer ratio was equal to 1 and 3 mol %, it was seen that the the drug transport mechanism changed with imprinting. When 5 mol % IA was used for both imprinted and non-imprinted hydrogels released drug with the same transport mechanism.

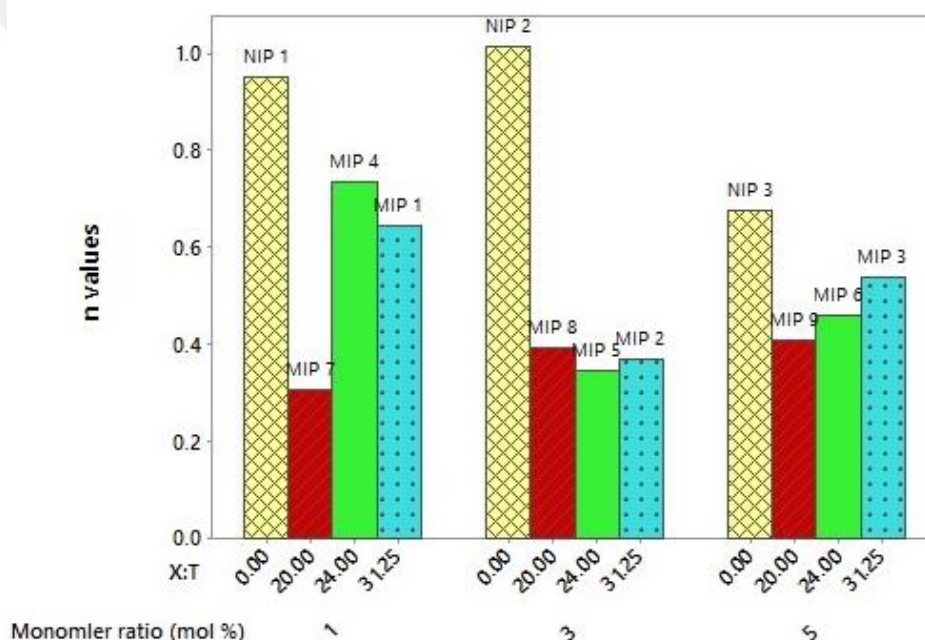


Figure 6.16 : n values DOX as a function of M:X and X:T ratios.

It can be seen from Figure 6.17, all imprinted networks (except MIP 1) had lower diffusion coefficients than non-imprinted counterparts. Below or equal to 0.45 for n value the diffusion was a rate-limiting process. When X:T was equal to 20 and 24 diffusion coefficients were lower than non-imprinted counterparts. Although hydrogels had high crosslinker ratio due to stability of the cavities, number of cavities which were created with high affinity were not sufficient above 24 (X:T=31.25) because of the TEGDMA-DOX and IA-TEGDMA interactions. Increasing monomer ratio to 5 mol % caused to an increase in diffusion coefficient significantly for both imprinted and non-imprinted hydrogels compared to hydrogels which had same X:T

ratios. This is strong evidence that the diffusion coefficients did not vary not only by functional monomer ratio and structural parameters of the network but also by the interactions of the template with arranged functional groups and crosslinker via imprinting technique.

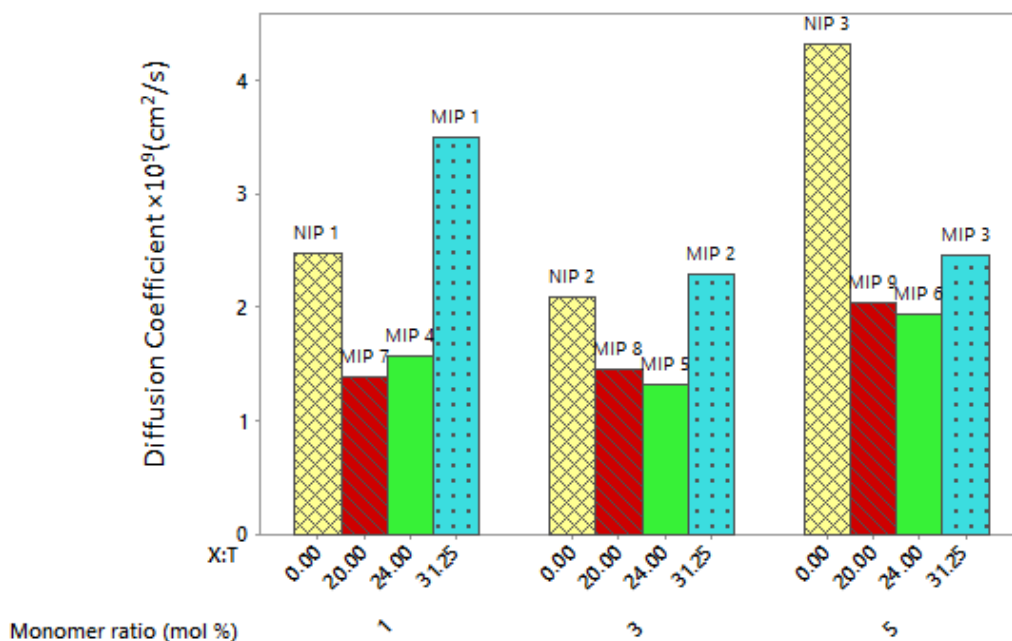


Figure 6.17 : Diffusion coefficients of the hydrogels as a function of M:X and X:T ratios.

Tumbling of the drug from one memory area to another led to slow diffusion coefficients and delayed release kinetics. A non-imprinted hydrogel matrix was not restricted to polymerize around the template during polymerisation and therefore had no macromolecular memory. On the other hand, the imprinting led to the generation of specifically imprinted binding sites. Diffusion path increased, unlike non-imprinted hydrogels, the drug molecules moved from one memory site to another for the imprinted hydrogels. Finally, this molecular binding actions were converted into controlled release and lower diffusion coefficients. It can be said that MIP 8 loaded and released the drug with best performance when the amounts of loaded, released DOX, the releasing control mechanism and diffusion coefficient were considered.

6.3 The Effect of Film Thickness on MIPs Performances and Structure Properties

Thickness of the hydrogel films effectively changed the loading properties of both MIPs and NIPs. Table 6.4 presented the hydrogel formulations of the paired thin and thick samples.

Table 6.4 : Hydrogel formulations of the selected hydrogels.

Functional monomer	Functional monomer ratio	Crosslinker	Crosslinker ratio	Drug ratio	Thin film code	Thick film code
IA	5	TEGDMA	5	0.250	MIP 1	MIP 18
IA	5	TEGDMA	5	-	NIP 1	NIP 18
IA	1	TEGDMA	5	0.160	MIP 9	MIP 17
IA	1	TEGDMA	5	-	NIP 3	NIP 17

As the thickness of the hydrogel films decreased, the amount of loaded drug on both the MIPs and NIPs increased. Also, the imprinted thin hydrogels were loaded greater amount of drug than the non-imprinted counterparts for thin films (Figure 6.18).

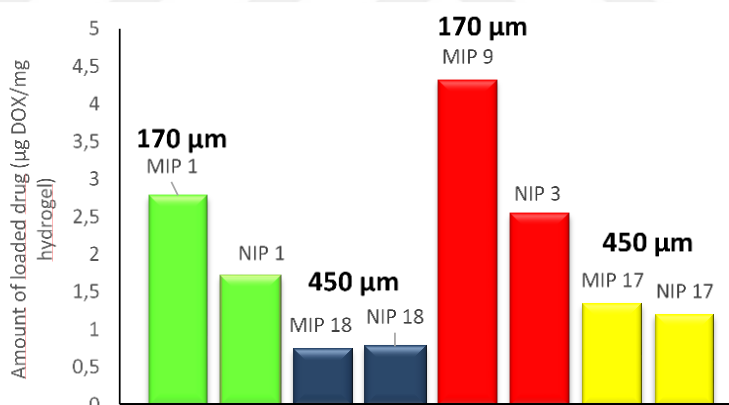


Figure 6.18 : Loading capacities of the hydrogels as a function of thickness.

If the volume fraction in the swollen state values were compared with thicker films (hydrogel 17 and 18), it can be clearly seen that $V_{2,s}$ values increased with decreasing the thickness of the hydrogels for both NIP and MIPs (Figure 6.19). On the other hand, although the hydrogels had same compositions their structural parameters were different from each other, swelling ratios increased (Figure 6.20).

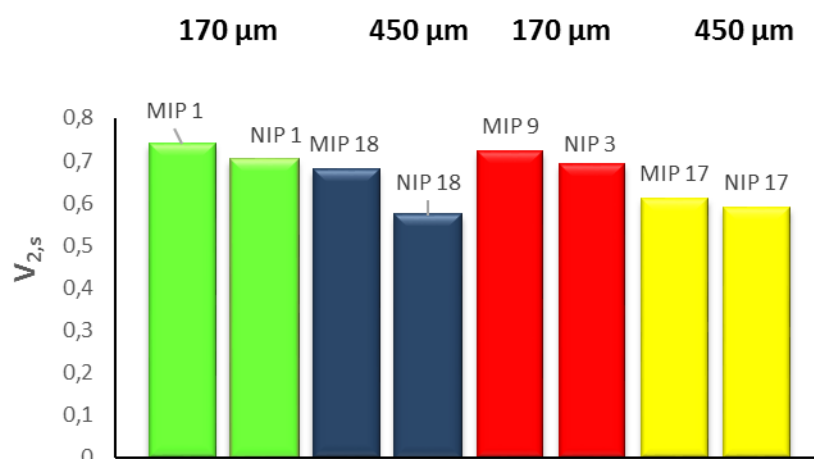


Figure 6.19 : Volume fraction in swollen states of the hydrogels as a function of thickness.

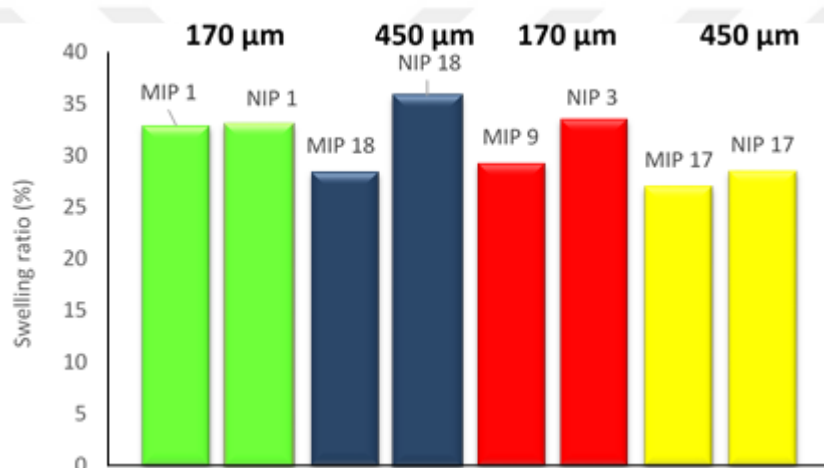


Figure 6.20 : Swelling ratios of the hydrogels as a function of thickness.

As we know, loading and releasing can be determined by two factors: the affinity of the drug to the imprinted cavities and the length of the diffusion path along the hydrogel network. We suggested that the decrease in the thickness of the films increased the affinity of the imprinted hydrogels to the DOX molecule by changing distribution of the functional monomer in the hydrogels. On the other hand, since drug molecules facilitate the entrance to hydrogel, it is more convenient to reach imprinted cavities and interact with functional groups. In this case, more drug molecules could interact with more number of the imprinted cavities. The other possibility is the shortening of the diffusion paths and by the fact that the drug molecules moved more easily in the hydrogel and settled in the cavities despite the high crosslinking structures.

In a similar study which was published by Yanez et al., they explored the effect of film thickness on swelling and loading capacity of the imprinted hydrogels [137]. They suggested that swelling ratio of the hydrogels that had the same composition increased with decreasing thickness of the films. Loading capacities were also slightly greater than thicker films for both non-imprinted and imprinted hydrogels. They attributed this case to the different functional monomer distribution in the hydrogels and diffusion path changes. These results support our finding in this regards.





7. CONCLUSIONS AND RECOMMENDATIONS

In this work, various imprinted and non-imprinted hydrogels were synthesized by modifying the ratio of components and reaction parameters. In this scope, optimization of the reaction conditions were studied and statistical analysis and modelling of the loading and release behavior of the hydrogels was explored in order to gain insight into interactions of template with other components present in the polymerization.

In the first stage of the study, the effect of polymerization method (UV and thermal polymerization) on the monomer conversion and reaction rate was investigated. The reaction rate and conversion were also calculated and in detail comparison was carried out. The application of thermal radicalic polymerization method resulted in high monomer conversion and similar results for both MIP and NIP was obtained, which indicated that polymerization type did not affect the imprinting efficiency. Thereafter, the initiator concentration, reaction time and reaction temperature was optimized in order to synthesize hydrogels with satisfactory conversion. This part was the important step in the development imprinting efficiency as well as enhancement hydrogel matrix design by using thermal radicalic polymerization in both imprinted and non-imprinted hydrogels. After optimization of polymerization conditions, DOX molecules were removed from hydrogel matrices with specific washing solutions and their loading abilities were compared with each other to select the optimum washing solution. 0.01 M oxalic acid: methanol:acetonitrile 65:15:20 (v:v:v) solvent mixture was chosen as first step washing mixture and methanol was selected as second step washing solvent.

All samples were thermally characterized by DSC. According to results, TEGDMA and IA based hydrogels exhibited low and high T_g values, respectively. These results indicated that flexibility of the polymer chains and affinity of the monomer to template molecule changed thermal properties of the hydrogels.

Swelling behaviors of the fabricated hydrogels were investigated at different mediums. Based on these results, increasing the pH of the medium improved swelling ratio of

the hydrogels. However, ionic strength due to the presence of NaCl in the release medium limited the water uptake properties of the hydrogels.

The thesis then focused on the analysis of drug release behavior of the imprinted hydrogels which had different monomer-crosslinker combinations with varying feed. The model parameters (factors) were selected (crosslinker type, crosslinker ratio, monomer type, monomer ratio and drug ratio present in the polymerization) based on the data in the literature. The statistical method was used to planning, organizing, analysing, and interpreting data from experiments. The wide range of parameters which can vary during synthesis of molecularly imprinted hydrogels required broad optimization of synthesis protocol. In our work, the composition of the polymerization mixture was selected using the statistical approach, which has proven its efficiency and becomes regularly used in many laboratories worldwide. Design of experiments (DOE) originated as a method to maximize the information obtained from experimental data. Applications of experimental design (DOE) in optimization of DOX-imprinted hydrogels were carried out using MINITAB software. ANOVA was employed to analyze loading and release performance of the hydrogels in order to confirm defensible results, statistically. Our study shed light to the design of improved imprinted hydrogels as well as to provide greater understanding of the origins of macromolecular structure for controlled release systems. The mechanism of imprinting response was developed by using statistical analysis methods. The data obtained from developed models indicated that IA and TEGDMA gave the best controlled DOX release properties to hydrogels. Three formulations were evaluated from response optimizer; they were synthesized and compared. Once the predicted and observed results were compared, it was seen that the results were consistent and the obtained models were successful. These results clearly demonstrated that DOE was a valid approach used here for the optimization of MIP hydrogels drug release performance.

The research showed that loading efficiency can be set by adjusting the hydrogel composition. Imprinted hydrogels synthesized with lower monomer ratio (1 mol %) and M:T ratio (4 or 6.25) demonstrated substantially lower affinity to DOX due to the insufficient amounts of functional monomer during the polymerization which not completely interact with the template to 'capture' all of its structure. On the other hand, a polymer fabricated with substantially higher ratios of functional monomer or

crosslinker (M:T or X:T generally was 31.25), demonstrated greater non-specific binding of the DOX molecules. In all these samples, nature of the monomer and crosslinker played a key role in achievement of the desired performances.

Our studies suggested that an optimal ratio of M:T, X:T, monomer/crosslinker ratio and also monomer/crosslinker nature should be determined to obtain a hydrogel with maximum DOX release performance. The transport mechanisms of the DOX loaded hydrogels was investigated by immersing hydrogels in 0.9 % NaCl solution. The amount of released DOX was mostly much more for imprinted hydrogels compared to their non-imprinted counterparts, because imprinting method transformed structural properties of the hydrogels including mesh size, polymer volume fraction in swollen state, swelling and affinity to drug molecules. The transport of DOX was diffusion and swelling limited for both imprinted and non-imprinted hydrogels. While non-imprinted hydrogels mostly released DOX with super case II transport, imprinted hydrogels released drug with non-Fickian diffusion mechanism. Release pattern of the fabricated drug delivery systems was also affected by the crosslinker and monomer nature and their ratios as loading studies.

The thickness was another important parameter on the imprinting efficiency. The lower the thickness of the hydrogels was, the lower the swelling ratio, mesh size, polymer volume fraction in swollen state and the slower the release rate and the higher imprinting efficiency. Hydrogels with 0.45 mm and 0.17 mm thickness were compared and it was observed that 0.17mm hydrogels released more amount of DOX than 0.45 mm hydrogels.

In summary, the synthesis of MIPs was a process involving several variables and its optimization was still a challenged task because of the interconnected nature of factors that affect the performance of MIPs. Therefore, for the development of MIP, it was preferred to have a method to reach to an appropriate hydrogel system for the template of interest. The results obtained by statistical and experimental approach were in consistence. The effects on the DOX release of the multiple interactions between the variables in the fabricated hydrogels was clearly recognized and explained.

The future study will be followed by a mutli-disciplinary approach by biologists, physicians, chemical engineers and chemists in order to develop contact lens and perform in vivo studies for the treatment of neovascularization.



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APPENDICES

APPENDIX A: FTIR spectra

APPENDIX B: NMR spectra

APPENDIX C: Calibration curve



APPENDIX A

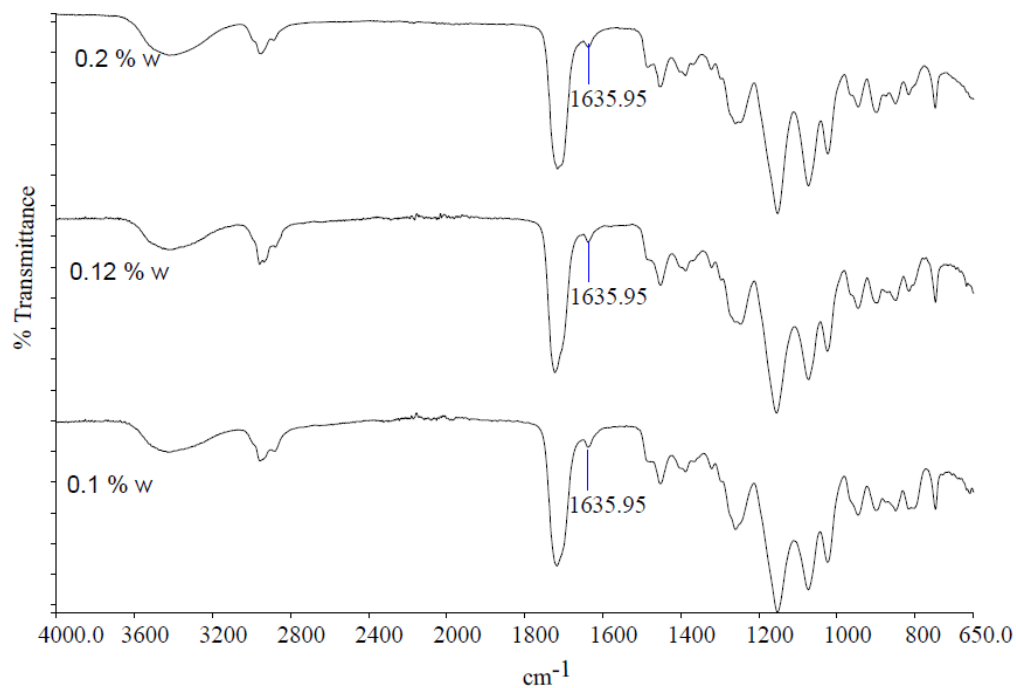


Figure A.1 : FTIR spectrums of hydrogels with different initiator concentrations.

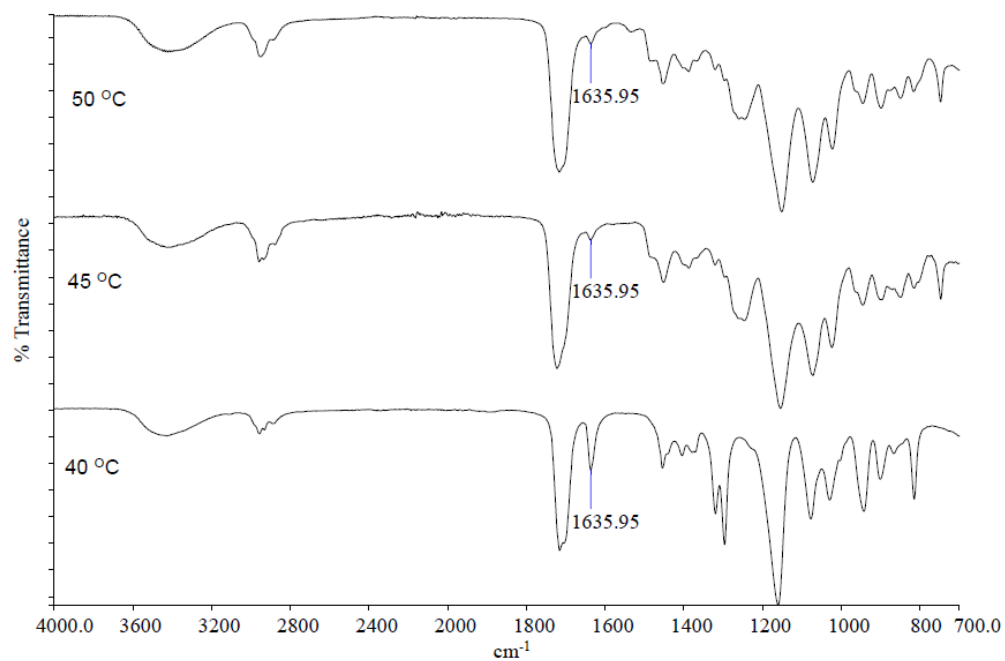


Figure A.2 : FTIR spectrums of hydrogels with different reaction temperatures.

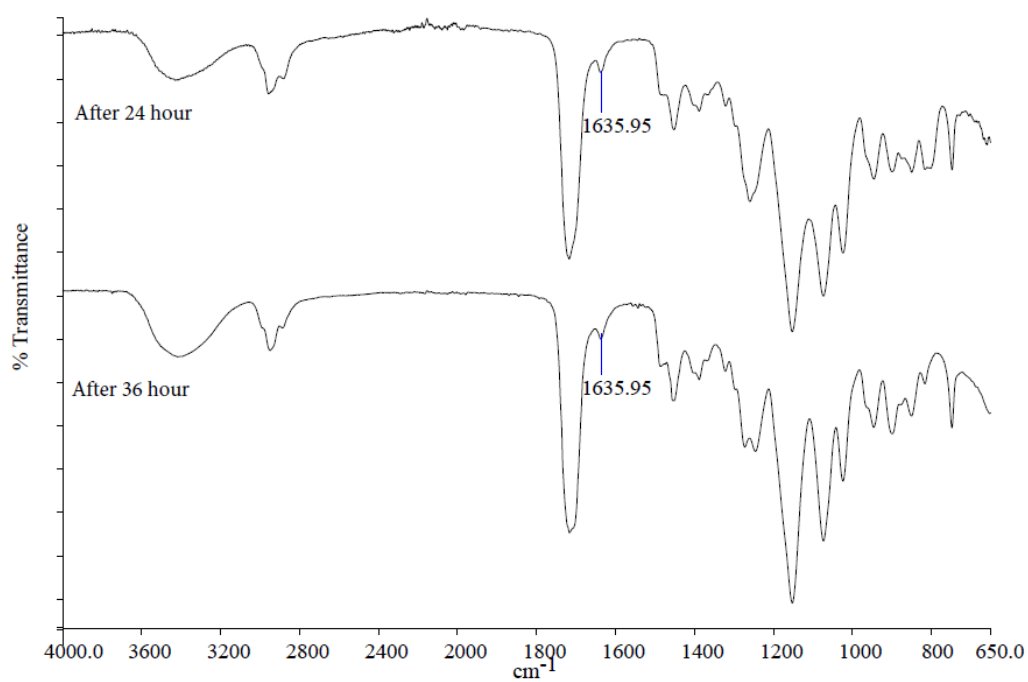


Figure A.3 : FTIR spectrums of hydrogels with different times.

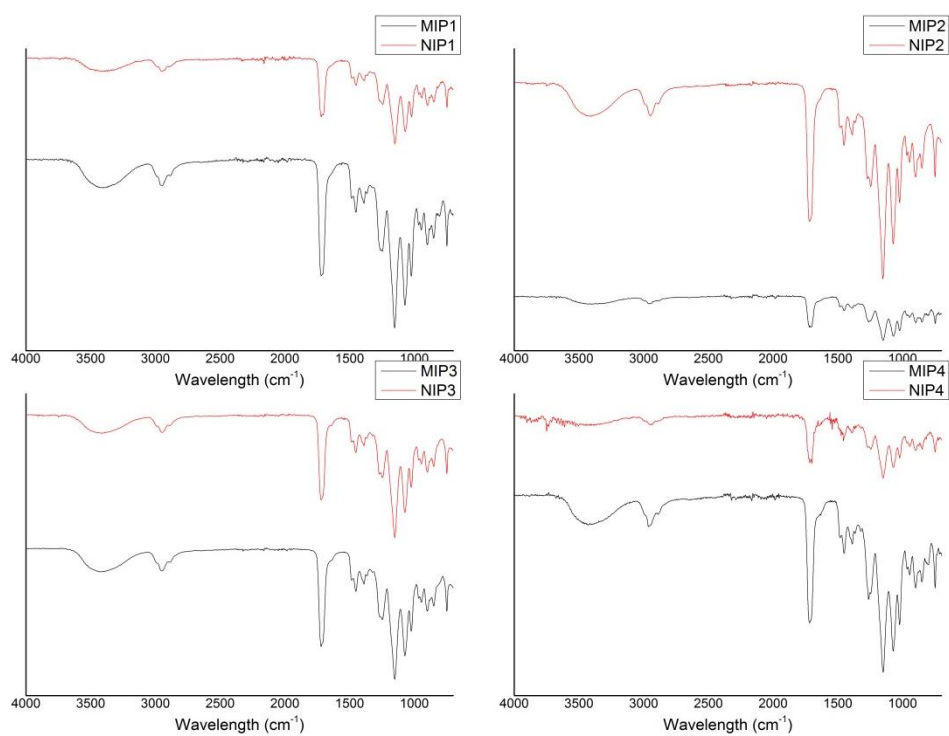


Figure A.4 : FTIR spectrums of imprinted and non-imprinted hydrogels 1-4.

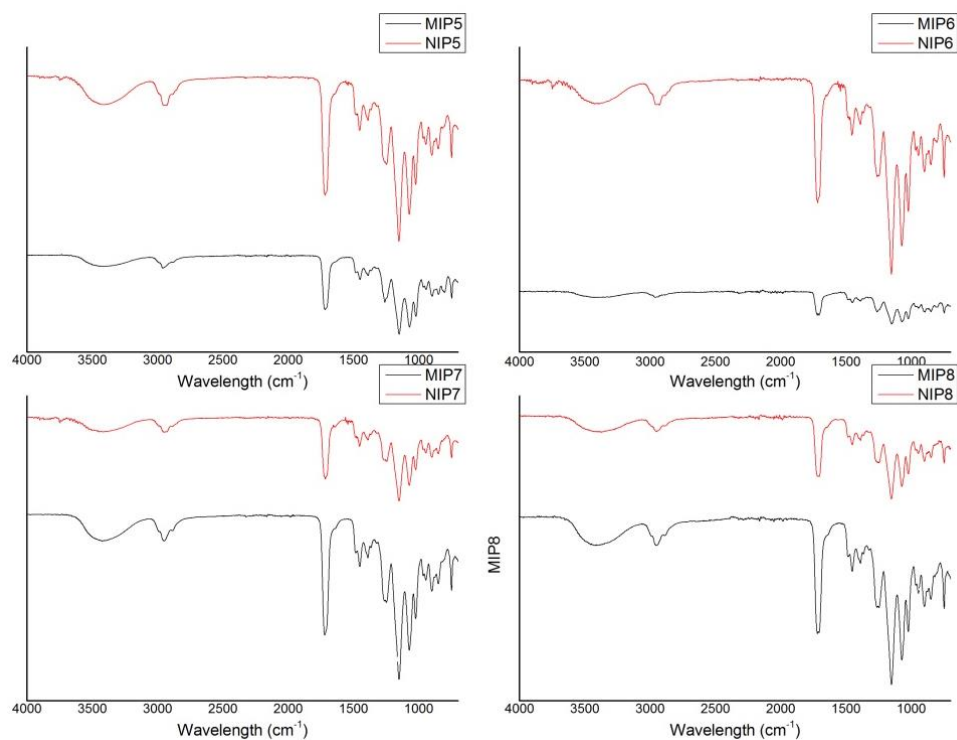


Figure A.5 : FTIR spectrums of imprinted and non-imprinted hydrogels 5-8.

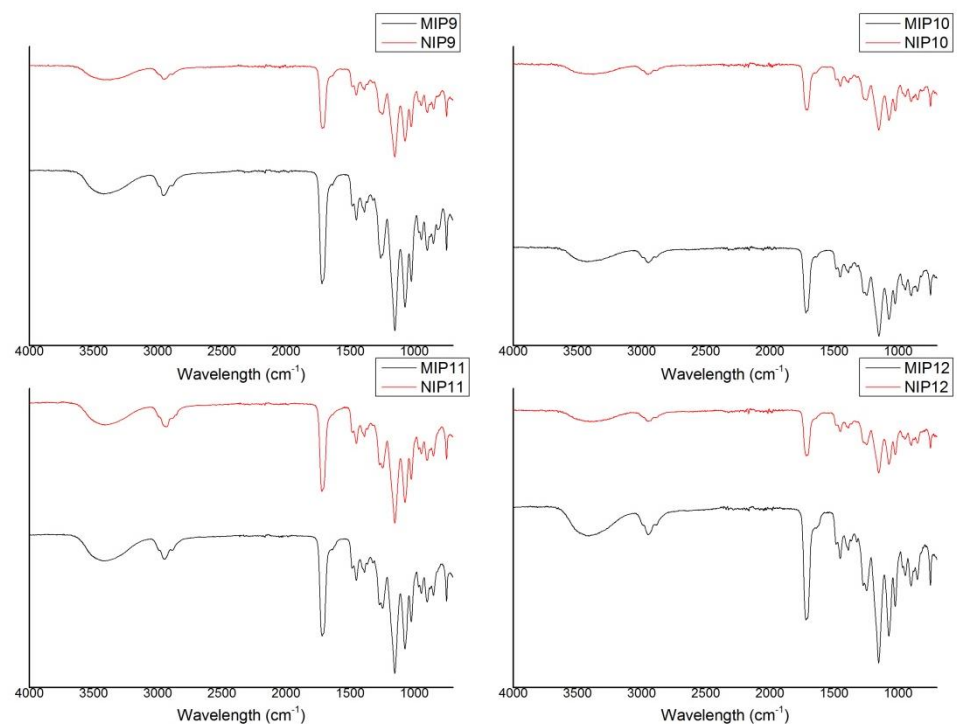


Figure A.6 : FTIR spectrums of imprinted and non-imprinted hydrogels 9-12.

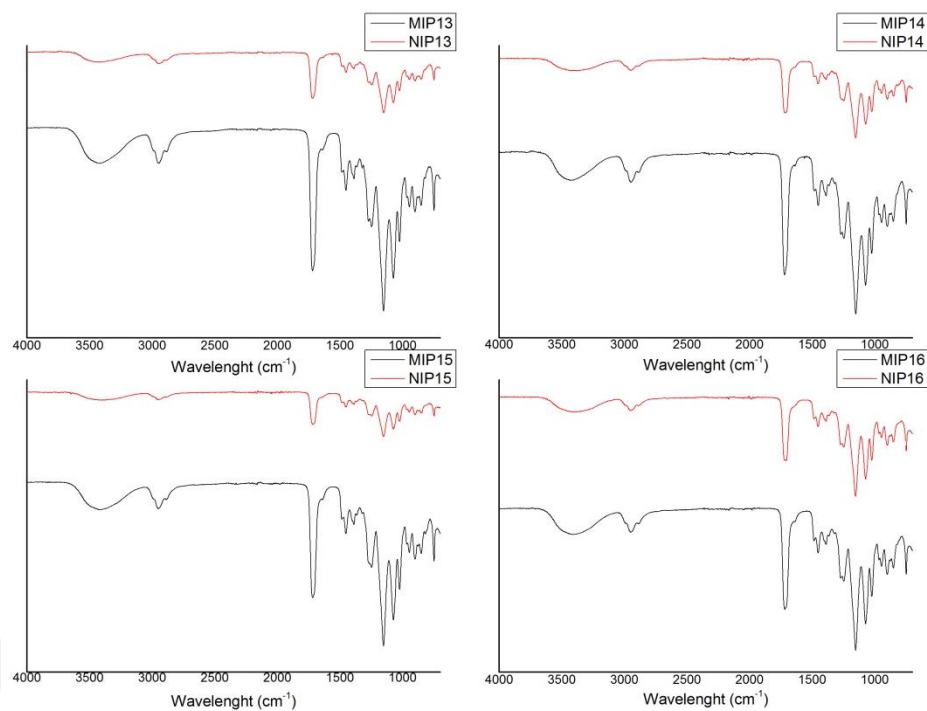


Figure A.7 : FTIR spectra of imprinted and non-imprinted hydrogels 13-16.

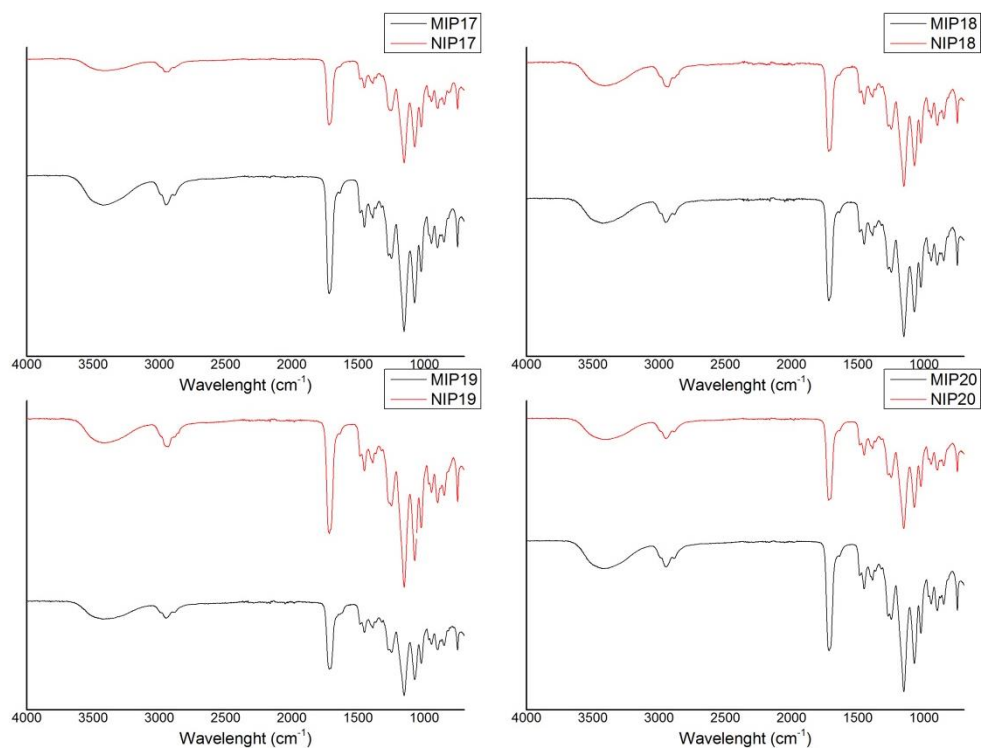


Figure A.8 : FTIR spectra of imprinted and non-imprinted hydrogels 17-20.

APPENDIX B

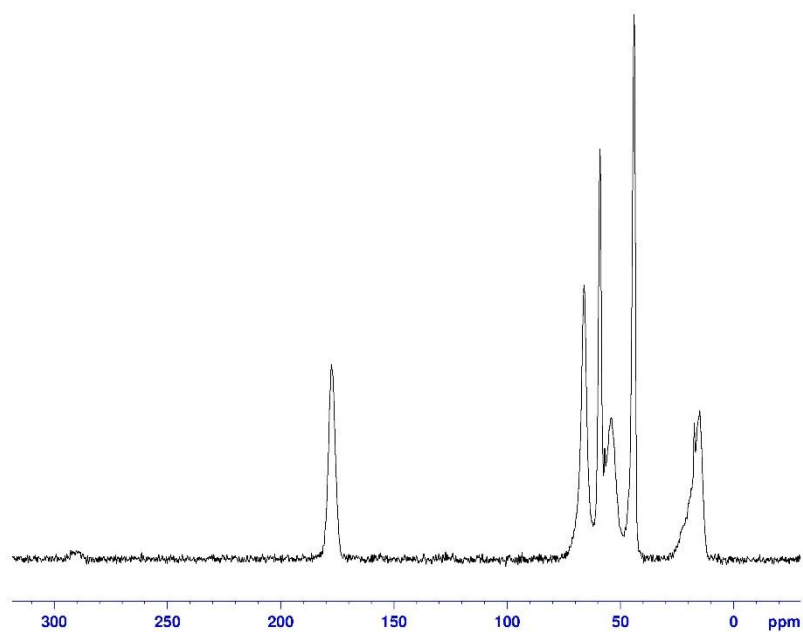


Figure B.1 : ^{13}C NMR for DOX-imprinted hydrogel.

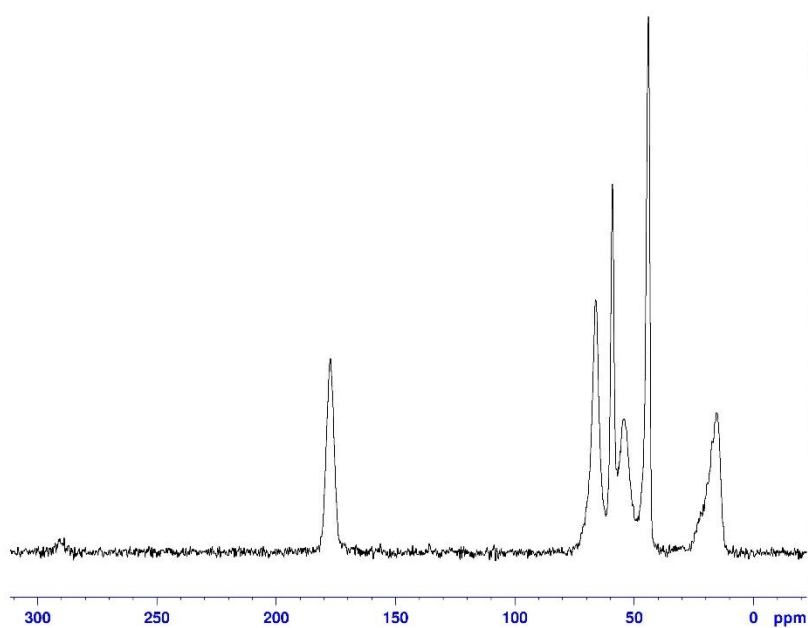


Figure B.2 : ^{13}C NMR spectra for non-imprinted hydrogel.

APPENDIX C

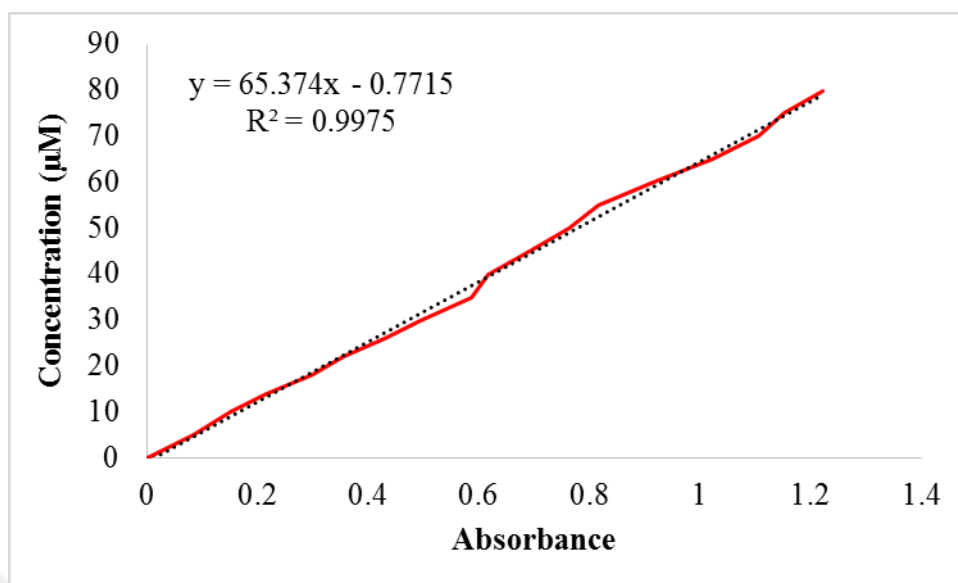


Figure C.1 : Calibration curve for drug loading and release studies.



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PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

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